Title: Publications from the Department of Agricultural and Biological Chemistry, the Pennsylvania State College, no. 219-227, 229-231, 233-237, 239-278, 280, 282-291

Place of Publication: State College, Pa.

Copyright Date: 1940-44

Master Negative Storage Number: MNS# PSt SNPaAg056.2

Publications from the Department
of
Agricultural and Biological Chemistry

The Pennsylvania State College
State College, Pa.

Volume 4

1940 - 1944

CONTENTS

219. Ward, R. E., Bechdel, S. I. and Guerrant, N. B.
1940. Carotene and Vitamin A in the Nutrition of Growing Dairy Cattle. Jour. Dairy Sci. 23(2):115-124.

220. Dutcher, R. Adams
1940. Research Activities of the Agr. Biochems. Penn State Alumni News 26(7):2-5.

221. Keith, T. B., Miller, R. C. and Henning, W. L.
1940. Soybean Oilmeal for Growing Lambs. Penna. State College Agr. Expt. Sta. Bulletin 397.

222. Haley, D. E.
1940. The Fertilizer Needs of Pennsylvania Tobacco. Eastern States Cooperator 16(5):5-7.

223. Frederick, Claire A., Guerrant, N. B., Dutcher, R. Adams and Knight, C. A.
1940. Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates. Proc. Soc. Exp. Biol. and Med. 44(1):203-207.

224. Knight, C. A., Dutcher, R. A., Guerrant, N. B. and Bechdel, S. I.
1940. Destruction of Ascorbic Acid in the Rumen of the Dairy Cow. Proc. Soc. Exp. Biol. and Med. 44(1): 90-93.

225. Matthews, Jason E., Jr., Dow, R. B. and Anderson, Arthur K.
1940. The Effects of High Pressure on the Activity of Pepsin and Rennin. Jour. Biol. Chem. 135(2):697-705.

226. Frear, Donald E. H. and Worthley, Harlan N.
1940. Deposition and Retention of Sprays on Apples. II. Penna. State College Agr. Expt. Sta. Bulletin 400.

227. Keith, T. B. and Miller, R. C.
1940. Levels of Protein for Pigs. Penna. State College Agr. Expt. Sta. Bulletin 401.

*228. Thorp, W. T. S., Rudy, W. J. and Boucher, R. V.
1940. A Nonspecific Lesion of the Esophagus and Oral Cavity of the Chicken. Amer. Jour. Veterinary Res. 1(1):78-82.

229. Miller, R. C., Keith, T. B., McCarty, M. A. and Thorp, W. T. S.
1940. Manganese as a Possible Factor Influencing the Occurrence of Lameness in Pigs. Proc. Soc. Exp. Biol. and Med. 45(1):50-51.

230. Guerrant, N. B. and Dutcher, R. Adams
1940. The Influence of Exercise on the Growing Rat in the Presence and Absence of Vitamin B_1. Jour. Nutrition 20(6):589-598.

231. Dow, R. B., Matthews, J. E., Jr. and Thorp, W. T. S.
1940. The Effect of High Pressure Treatment on the Physiological Activity of Insulin. Amer. Jour. Physiol. 131(2):382-387.

**232. Lisse, Martin W.
1940. Biocolloids. Edwards Bros., Inc., Ann Arbor, Michigan. 2nd Edition. v + 318 pages.

* No reprints available

** Book - bound separately

233. Dutcher, R. Adams
1941. Book Review of "Essentials of Nutrition" by H. C. Sherman and C. S. Lanford. Jour. Chem. Education 18(2):99.

234. Miller, R. C. and Keith, T. B.
1941. The Optimum Level of Protein Intake for the Growth and Fattening of Swine. Jour. Nutrition 21(4): 419-429.

235. Keith, T. B., Miller, R. C. and McCarty, M. A.
1941. Levels of Supplementary Protein for Pigs on Pasture. Penna. State College Agr. Expt. Sta. Bulletin 407.

236. Ziegler, P. T., Bentley, F. L. and Miller, R. C.
1941. Alfalfa-Molasses Silage as a Roughage for Fattening Steers. Penna. State College Agr. Expt. Sta. Bulletin 410.

237. Dutcher, R. Adams
1941. Recent Developments in Vitamin Research. Feedstuffs 13(26):11-15.

*238. Stadelman, W. J., Murphy, R. R., Callenbach, E. W. and Boucher, R. V.
1941. Dry Skim Milk in Ring-necked Pheasant Chick Rations. Game Breeder & Sportsman 46(6):102-103, 114-116.

239. Gribbins, M. F., Reid, J. J. and Haley, D. E.
1941. The Distribution of Potassium in Bright Leaf Cigarette Tobacco and Its Influence on the Quality of the Leaf. Jour. Agr. Res. 63(1):31-39.

* No reprints available

240. Knight, C. A., Dutcher, R. A., Guerrant, N. B. and Bechdel, S. I.
1941. Utilization and Excretion of Ascorbic Acid by the Dairy Cow. Jour. Dairy Sci. 24(7):567-577.

241. Dutcher, R. Adams
1941. New Science Building at Pennsylvania State College. Ind. and Eng. Chem. Anal. Ed. 13(7): 512-514.

242. Boucher, R. V.
1941. Why Eggs Are Classified as a Protective Food. U. S. Egg and Poultry Magazine 47(8):470-473, 512.

243. Miller, R. C. and Keith, T. B.
1941. Manganese in Animal Nutrition With Special Reference to Pigs. Feedstuffs 13(35):14-15.

244. Stadelman, W. J., Murphy, R. R., Callenbach, E. W. and Boucher, R. V.
1941. A Comparison of Pheasant Starting Rations Under Practical Commercial Conditions. Penna. Game News 12 (5):4-5, 28-29.

245. Patrick, H., Boucher, R. V., Dutcher, R. Adams and Knandel, H. C.
1941. Biotin and Prevention of Dermatitis in Turkey Poults. Proc. Soc. Exp. Biol. and Med. 48(2): 456-458.

246. Thorp, W. T. S., Keener, H. A., Bechdel, S. I. and Guerrant, N. B.
1942. Observations on the Pathology of Dairy Calves on Low Vitamin A Diets. Amer. Jour. Veterinary Res. 3(6): 27-31.

247. Thorp, W. T. S., Shigley, J. F. and Anderson, A. K.
1942. Sulfapyridine in the Treatment of Calf Pneumonia. Jour. Amer. Veterinary Med. Assoc. 100(780): 225-228.

248. Reid, J. J., Naghski, J., Farrell, M. A. and Haley, D. E.
1942. Bacterial Leafspots of Pennsylvania Tobacco. I. Occurrence and Nature of the Microorganism Associated with Wildfire. Penna. State College Agr. Expt. Sta. Bulletin 422.

249. Ziegler, P. T. and Miller, R. C.
1942. Methods of Hastening Absorption of Salt in Farm-Cured Ham. Penna. State College Agr. Expt. Sta. Bulletin 427.

250. Keith, T. B., Miller, R. C., Thorp, W. T. S. and McCarty, M. A.
1942. Nutritional Deficiencies of a Concentrate Mixture Composed of Corn, Tankage, Soybean Oilmeal, and Alfalfa Meal for Growing Pigs. Jour. Animal Sci. 1(2):120-125.

251. Chornock, Charlotte, Guerrant, N. B. and Dutcher, R. Adams
1942. Effect of Manganese on Calcification in the Growing Rat. Jour. Nutrition 23(5):445-458.

252. Ott, Walther H., Boucher, R. V. and Knandel, H. C.
1942. Feeding-Cane Molasses as a Constituent of Poultry Rations. I. Molasses for Growing Chickens. Poultry Sci. 21(4):340-345.

253. Worthley, H. N. and Frear, D. E. H.
1942. Deposition of Lead from Lead Arsenate Spray Mixtures and Its Retention on Pyralin Plates and Apple Leaves and Fruits. Jour. Econ. Entomology 35(2):205-210.

254. Keener, H. A., Bechdel, S. I., Guerrant, N. B. and Thorp, W. T. S.
1942. Carotene in Calf Nutrition. Jour. Dairy Sci. 25(7):571-583.

255. Zipkin, Isadore, Anderson, Arthur K. and Thorp, W. T. S.
1942. A Study of the Administration of Sulfanilamide to the Normal Dairy Cow. Amer. Jour. Veterinary Res. 3(9):329-335.

256. Ott, Walther H., Boucher, R. V. and Knandel, H. C.
1942. Feeding-Cane Molasses as a Constituent of Poultry Rations. II. Molasses for Adult Chickens. Poultry Sci. 21(6):536-539.

257. Haley, D. E. and Reid, J. J.
1943. The Bearing of Potassium on the Quality of Tobacco. Soil Sci. 55(1):79-85.

258. Boucher, Robert V. and Haley, D. E.
1943. Soil Composition Has Direct Relation to Animal Health. Mineral Nutrients of Farm Crops Show Variations Throughout Country. Feed Bag Annual "Red Book", pp. 111-113.

259. Duisberg, P. C. and Miller, R. C.
1943. Relation of Hydrogen-Ion Concentration to Color Developed in Cured Pork. Food Research 8(1): 78-87.

260. Haley, D. E., Street, O. E., Farrell, M. A. and Reid, J. J.
1943. The Yield and Composition of Cigar-leaf Tobacco as Influenced by Fertilizer and Preceding Crop. Penna. State College Agr. Expt. Sta. Bulletin 440.

261. Miller, R. C.
1943. Wartime Problems of Feeding and Nutrition. Jour. Amer. Veterinary Med. Assoc. 102(793):300-303.

262. King, H. L. and Frear, D. E. H.
1943. Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to *Tetranychus telarius* (L.). Jour. Econ. Entomology 36 (2):263-265.

263. Patrick, H., Boucher, R. V., Dutcher, R. Adams and Knandel, H. C.
1943. Prevention of Perosis and Dermatitis in Turkey Poults. Jour. Nutrition 26(2):197-204.

264. Miller, R. C., Keith, T. B., Thorp, W. T. S. and McCarty, M. A.
1943. Nutritive Requirements of Young Pigs. Penna. State College Agr. Expt. Sta. Bulletin 449.

265. Frear, Donald E. H. and Anthony, R. D.
1943. The Influence of Date of Sampling on the Value of Leaf Weights and Chemical Analyses in Nutrition Experiments with Apple Trees. Proc. Amer. Soc. Hort. Sci. 42: 115-122.

266. Miller, R. C., Pence, J. W., Dutcher, R. A., Ziegler, P. T. and McCarty, M. A.
1943. The Influence of the Thiamine Intake of the Pig on the Thiamine Content of Pork with Observations on the Riboflavin Content of Pork. Jour. Nutrition 26(3):261-274.

267. Dutcher, R. Adams
1943. Some New Aspects of Vitamin A Chemistry. Record of Chemical Progress 4(3 & 4):50-53.

268. Keith, T. B., Miller, R. C., McCarty, M. A. and Thorp, W. T. S.
1943. Feed Mixtures for Young Pigs. Penna. State College Agr. Expt. Sta. Bulletin 456.

269. Vogel, R. C. and Lisse, M. W.
1943. pH Measurements on Thixotropic Gel Systems Using the Glass Electrode. Jour. Physical Chem. 47(9):678-685.

270. Frear, Donald E. H.
1943. A Catalogue of Insecticides and Fungicides. Science 98(2557):585.

271. Stadelman, W. J., Murphy, R. R., Callenbach, E. W. and Boucher, R. V.
1944. Satisfactory War-Time Rations for Pheasant Chicks. Jour. Wildlife Management 8(1):56-60.

272. Frear, Donald E. H.
1944. Deposition and Retention of Sprays. III. Apparatus and Methods for Laboratory Spraying. Penna. State College Agr. Expt. Sta. Bulletin 463.

273. Alderfer, R. B., Gribbins, M. F. and Haley, D. E.
1944. Effect of Waste Sulfite Liquor on Aggregation of Soil Particles. Ind. and Eng. Chem. 36(3):272-274.

274. Clopton, J. R. and Triebold, H. O.
1944. Fanweed Seed Oil. Potential Substitute for Rapeseed Oil. Ind. and Eng. Chem. 36(3):218-219.

275. Miller, H. J., Frear, D. E. H., King, H. L. and Fagan, F. N.
1944. Performance of New Spray Equipment. Proc. Penna. Hort. Assoc. 21(1):59-64.

276. Dills, L. E., Frear, D. E. H. and King, H. L.
1944. Cabbage Maggot Tests in 1943. Penna. Vegetable Growers' News 9(1):42-45.

277. Boucher, Robert V.
1944. Efficacy of Vitamin D from Different Sources for Turkeys. Jour. Nutrition 27(5):403-413.

278. Frear, D. E. H., Miller, H. J. and Fagan, F. N.
1944. Comparative Spray Deposits and Scab Control from Speed Sprayer and Single-Multiple Gun Sprayer. Proc. Amer. Soc. Hort. Sci. 44:129-133.

*279. Fagan, F. N., Frear, D. E. H. and Miller, H. J.
1944. Speed Sprayer Is Big Labor Saver; Works Best on Medium High Trees. Science for the Farmer, Supplement No. 3 to Bulletin 446, the 56th Annual Report, Penna. State College Agr. Expt. Station.

* No reprints available

280. Clopton, John R. and Triebold, Howard O.
1944. Fanweed, A Potential Major Source of Vegetable Oil. The Chemurgic Digest 3(12):160-161.

*281. Clopton, J. R. and Triebold, H. O.
1944. Spare the Weed. Industrial Bulletin of Arthur D. Little, Inc., No. 203.

282. Quinones, Victor L., Guerrant, N. B. and Dutcher, R. Adams
1944. Vitamin Content of Some Tropical Fruits, Their Juices and Nectars. Food Research 9(5):415-417.

283. Althouse, Paul M. and Triebold, Howard O.
1944. Physical Constants of Methyl Esters of Commonly Occurring Fatty Acids. Vapor Pressure. Ind. and Eng. Chem. Anal. Ed. 16(10):605-606.

284. King, H. L. and Frear, D. E. H.
1944. Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity as Fumigants. Jour. Econ. Entomology 37(5):629-633.

285. King, H. L. and Frear, D. E. H.
1944. Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity Against _Tribolium confusum_. Jour. Econ. Entomology 37(5):634-637.

286. King, H. L., Frear, D. E. H. and Dills, L. E.
1944. Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to _Aphis rumicis_. Jour. Econ. Entomology 37(5):637-640.

* No reprints available

287. Dills, L. E., Frear, D. E. H. and King, H. L.
1944. Mercury Substitutes for Cabbage Maggot Control. Jour. Econ. Entomology 37(5):640-643.

288. Whitlock, Gaylord P., Guerrant, N. B. and Dutcher, R. Adams
1944. Response of Rats to Diets Containing Propylene Glycol and Glycerol. Proc. Soc. Exp. Biol. and Med. 57(1):124-125.

289. Gribbins, M. F., Haley, D. E. and Reid, J. J.
1944. The Fermentation of Cigar-leaf Tobacco as Influenced by the Addition of Yeast. Jour. Agr. Res. 69(9):373-381.

290. Ott, Walther H. and Boucher, R. V.
1944. Lactalbumin as a Protein Supplement for Growing Chickens. Poultry Sci. 23(6):497-506.

291. Denko, Charles W. and Anderson, Arthur K.
1944. Studies on the Toxicity of Gold Compounds in Rats. Jour. Lab. Clin. Med. 29(11):1168-1176.

*292. Boucher, Robert V.
1944. Source of Vitamin D Determines Amount of Supplement Required in Turkey Feed. Science for the Farmer, Supplement No. 1 to Bulletin 464, the 57th Annual Report, Penna. State College Agr. Expt. Station.

* No reprints available

Reprinted from JOURNAL OF DAIRY SCIENCE, February, 1940, Vol. XXIII, No. 2, pages 115–124.

CAROTENE AND VITAMIN A IN THE NUTRITION OF GROWING DAIRY CATTLE[1]

R. E. WARD AND S. I. BECHDEL
Department of Dairy Husbandry
AND
N. B. GUERRANT
Department of Agricultural and Biological Chemistry,
The Pennsylvania State College

Jones, Eckles and Palmer (10) were among the first investigators to demonstrate the necessity of vitamin A for the proper nutrition of dairy calves. Bechdel, Eckles, and Palmer (1), and Converse and Meigs (3, 4, 12) submitted further evidence that calves require carotene or vitamin A. Hart and Guilbert (9) observed vitamin A deficiency in range cattle under natural conditions. Guilbert and Hart (7) found that about twenty-nine micrograms of carotene as it exists in alfalfa hay per day per kilogram of body weight prevented or cured vitamin A deficiency in cattle. Guilbert, Miller, and Hughes (8) working with cattle, sheep, and swine confirmed the above findings and reported that 25 to 30 micrograms of carotene daily per kilogram body weight, or 6 to 8 micrograms daily of vitamin A was just sufficient to prevent night blindness. Kuhlman, Gallup, and Weaver (11) found a commercial preparation of carotene dissolved in cottonseed oil to be an effective source of vitamin A for calves. Soldatenkiv (15) stated that feeding carrots to calves apparently accelerated recovery from respiratory diseases and built up resistance to infection.

In connection with investigations at the Pennsylvania Experiment Station (5) calves have been observed to become blind and to exhibit other symptoms of vitamin A deficiency while receiving levels of carotene intake (from timothy hay) in excess of that stated by Guilbert and Hart (7). Further investigations were, therefore, undertaken with the object of securing additional information regarding the carotene requirements of growing dairy cattle and to study the utilization of carotene from different sources of feedstuffs.

METHOD OF ESTIMATING THE CAROTENE CONTENT OF FEEDSTUFFS

For the determination of carotene in the feedstuffs the Struve modification of the Guilbert method (6) was used. This method differed from the

---

Received for publication June 28, 1939.

[1] Authorized for publication on June 23, 1939, as paper No. 911 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The data presented in this paper are taken from a thesis submitted by Ruel E. Ward to the Graduate School of The Pennsylvania State College in partial fulfillment of requirements for the degree of Doctor of Philosophy.

Guilbert procedure in that half saturated methyl alcoholic-potassium hydroxide was used for the digestion and saponification, and also in that absolute methyl alcohol was added to the ethyl ether solution and then the ether evaporated off on a water bath at 65° C. instead of evaporated to dryness in vacuo as in the Guilbert method. The final petroleum ether solution of carotene was compared to a standard potassium dichromate solution.

In order to check on the accuracy of this method of carotene estimation, biological assays were also made on a number of samples of the hays. The results indicated a satisfactory agreement between the values obtained by the two methods of assay.

GROWTH OF CALVES RECEIVING DIFFERENT LEVELS OF CAROTENE INTAKE

The object of this phase of the investigation was to compare the effect of different levels of carotene intake on the growth and well-being of young calves. Grade Holstein calves (ten bulls and three heifers) were used as the experimental subjects. Mow-burned brown alfalfa hay containing only 1.3 micrograms of carotene and dehydrated alfalfa containing 65 micrograms of carotene per gram were used as sources of carotene. These hays were mixed in such proportions that mixtures containing 1.3, 4, 9, 17, and 65 micrograms of carotene per gram were available for the feeding tests.

The calves were housed in a modern barn which was equipped with a heating and ventilating system. This equipment made it possible to maintain fairly uniform experimental conditions throughout the winter months, and prevented barn temperatures lower than 28° F. During the summer months the calves were turned out in a dry lot for several hours each day. A concentrate mixture, made up of feeds of low vitamin A content, but containing 11.3 per cent digestible crude protein and 69.9 per cent total digestible nutrients was used. The constituents were wheat bran, "Banner" oat feed, barley, linseed oil meal, molasses, salt, and irradiated yeast. All calves were fed whole milk during the first three weeks on experiment, after which skimmilk was fed. The concentrate mixture was supplied to the animals according to their appetites. Water and salt were supplied ad libitum. The hay was kept before the calves at all times, but the animals were usually 40 to 50 days old before appreciable amounts were consumed.

The carotene contents of the various hay mixtures fed as well as gains in body weight of the respective animals are shown in figure 1. While gain in weight of the animals was the chief criterion used to determine adequacy of carotene intake, close observations were made for other symptoms of vitamin A deficiency. The carotene content of the blood serum was run at two-week intervals. The Palmer method as modified by Connor (2) and later by White and Gordon (16) was used for these blood carotene determinations.

DATA

In order to conserve space, the data obtained in this phase of the investigation have been reduced to graphic form and are presented in figure 1.

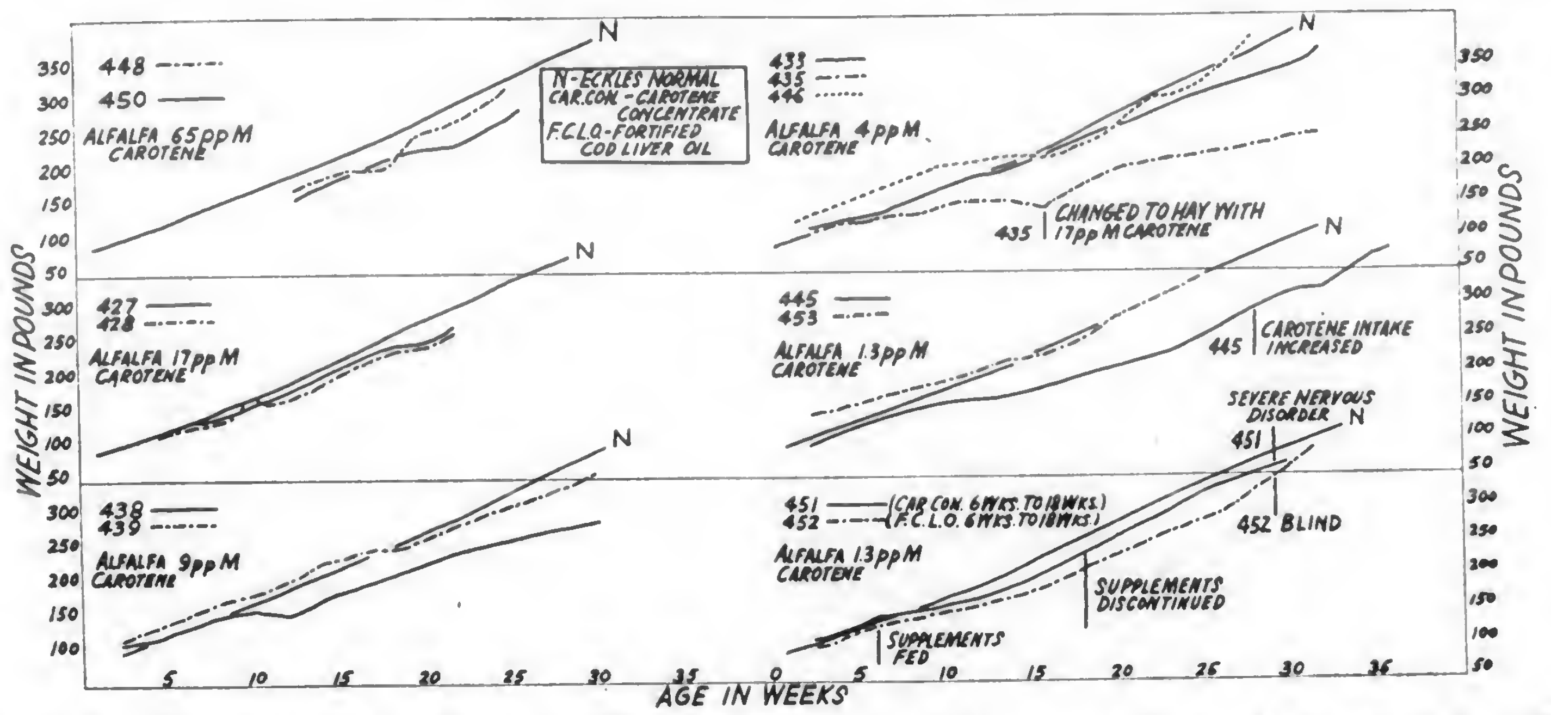


FIG. 1. Growth of calves while receiving equal quantities of hays of different carotene contents.

DISCUSSION

Examination of the data presented in figure 1 show clearly that increases of carotene intake within the limits of this experiment did not result in increased growth of the calves. Even at levels of intake much below the reported minimum the growth continued normal for some time and was not greatly affected until the avitaminosis A reached an advanced stage. This may suggest that decreased growth rate frequently reported may be caused by intestinal, respiratory or other disturbances which so frequently accompany vitamin A deficiency. The average growth rate of two calves (Calves 448 and 450) which received the hay containing 65 micrograms of carotene per gram was no greater than the growth rate of calves (Calves 445 and 453) which received the hay containing only 1.3 micrograms, although this latter hay supplied only 4 to 8 micrograms of carotene per pound body weight per day. Several of the calves that received the carotene poor hay exhibited other symptoms of vitamin A deficiency. Distinct papillitis was observed in the eyes of calves 445 and 453 when they were several months of age. Calf 453 developed night blindness when approximately six months of age and calf 445 manifested several of the spasms which occasionally accompany vitamin A deficiency. Calf 452 was given from 0.6 to 0.8 gram of fortified cod liver oil daily and calf 451 was given from 0.6 to 0.8 gram of a carotene supplement for several months after they were placed on experiment. These supplements raised the vitamin A intake of the two calves to an equivalent of 10 to 12 micrograms of carotene per pound body weight per day. When these supplements were discontinued, calf 452 became permanently blind in about eleven weeks and calf 451 developed a severe nervous disorder. The administration of the carotene concentrate at this time was not effective in promoting any noticeable improvement in the condition of calf 451 while calf 452 did show a slight improvement in general condition.

With the exception of calf 435 which did not consume much hay (0.6 pound per 100 pounds body weight per day), the hay with only 4 micrograms of carotene per gram was just as satisfactory as hays of higher carotene content in preventing vitamin A deficiency and in promoting growth. For these calves this hay supplied approximately twenty micrograms of carotene per pound live weight per day. No immediate benefit was observed as the result of feeding hays of higher carotene content.

Calves 445, 453, 451, and 452, that received the hay containing only 1.3 micrograms of carotene per gram, always maintained less than .01 milligram of carotene per 100 cc. of blood serum. When calves 451 and 452 received their respective vitamin A supplements, the blood serum contained .01 to .015 milligram carotene per 100 cc. serum. Calves 443 and 446 had blood serum values ranging from .01 to .02 milligram when fed hay containing 4 micrograms of carotene per gram. In the case of those calves fed hays with higher carotene content, the minimum blood value was .02 milligram per 100 cc. of serum. It is of interest to note that the blood serum value of calf 435 dropped to approximately .0025 milligram per 100 cc. When hay containing 17 micrograms per gram was fed, the blood carotene rose very rapidly to a level of .03 milligram per 100 cc. of serum. The blood serum of all calves usually dropped appreciably for the first few weeks after birth. After this period there was a general increase in blood serum carotene as the age advanced even at minimum levels of carotene feeding.

AVAILABILITY OF CAROTENE FROM DIFFERENT SOURCES

This phase of the investigation was designed with the view of determining the biological value of carotene from different plant sources. In these studies, twelve Holstein and seven Guernsey calves were used as experimental subjects. These animals were so distributed in the different experimental groups as to permit a comparison of the carotene requirements of the two breeds.

The concentrate mixture used was identical with that employed in the previous experiment. All but three of the calves received whole milk during the first few weeks of the experiment, after which they received skimmilk until approximately five months of age. Three calves, two Holstein and one Guernsey, were changed to skimmilk within a few days after birth. Two of these were supplied with a commercial carotene supplement and one calf was supplied with fortified cod liver oil. The following feedstuffs were used as sources of carotene: alfalfa hay, timothy hay, alfalfa molasses silage, corn silage, yellow corn meal, and carotene concentrate (carotene in cottonseed oil). A fortified cod liver oil was also fed as a source of vitamin A. Where the amount of hay fed was limited or where no hay was fed additional roughage was supplied to the calves in the form of beet pulp or wheat straw. An attempt was made to keep the calves growing and in the best possible state of health. All animals were weighed at weekly intervals and these weights were used in calculating the carotene and the food requirements of the respective animals for the succeeding week.

In these studies, changes in the fundus of the eye were used as a criterion of vitamin A deficiency. These changes were observed by means of an ophthalmoscope used according to the procedure reported by Moore (13, 14). Following a period in which the calves received a sub-minimal vitamin A intake, papillitis as well as other changes in the eyes appeared. These changes could be readily detected as they appeared but some difficulty was experienced in determining just when the calf was again receiving sufficient vitamin A. It had been observed in earlier work at this station (5) that the pupil of the calf's eye became dilated and lost its ability to contract on exposure to a bright light when the animal had been receiving a vitamin A deficient diet. When sufficient carotene or vitamin A was supplied to such calves, the pupil usually recovered its ability to contract. Therefore, in these studies, the dilation of the pupil was also used as a measure of vitamin A deficiency. In addition, the appearance of night blindness, digestive disturbances, respiratory disturbances, and the carotene of the blood were considered in evaluating results.

The calves were supplied carotene from one of the above mentioned sources at a definite level for several weeks. If no deficiency was noted the level of carotene intake was lowered progressively until a deficiency was observed. The level of carotene intake was then raised progressively until the deficiency symptoms disappeared.

RESULTS

The data obtained concerning the requirement of individual calves for carotene from the different sources have been summarized and are presented

TABLE 1

Level of carotene intake above which no symptoms of vitamin A deficiency were observed

Feedstuff	Micrograms per pound body weight daily	
	Holstein	Guernsey
Timothy hay	19	21
Alfalfa hay	14	15
Alfalfa molasses silage	33	......
Corn silage	17	......
Carotene concentrate	11	13
Yellow corn meal	18	......
Fortified cod liver oil* (vitamin A)	13	13
Fortified cod liver oil plus hay*	23	19

* Calculated by assuming one U.S.P. unit equal to 0.6 *gamma* of carotene.

in table 1. The data are expressed as the number of micrograms of carotene per pound body weight per day required to cure or to prevent vitamin A deficiency. The micrograms of vitamin A from fortified cod liver oil are calculated from the manufacturers guaranteed minimum by assuming that one U.S.P. unit is equal to 0.6 *gamma* of carotene.

In table 2 the amounts of carotene in the blood serum of the above calves while receiving the various carotene intakes are presented.

TABLE 2

Blood serum carotene in relation to carotene intake

Daily carotene intake***	Mean blood serum carotene**					
	5–10	10–15	15–20	20–30	30–50	50–400
Source of carotene						
Timothy hay	Trace	.005	.008	.015		
Alfalfa hay	Trace	.006	.008	.015	.03	.03–.22
Alfalfa molasses silage		.004	.014	.015	.01	
Corn silage		.005	.006	.008		
Carotene concentrate	Trace	.009				
Yellow corn meal			.014			
Fortified cod liver oil		.005	.01			
Fortified cod liver oil plus hay		.003	.005			
Average of all feeds	Trace	.0053	.008	.014	.02	.03–.22

** Mgms. per 100 cc. serum.
*** Micrograms per pound body weight.

In eight cases (four Holsteins and four Guernseys) respiratory and intestinal disturbances appeared when calves were receiving sufficient carotene to prevent papillitis. The four Holstein calves made a favorable recovery when their carotene intakes were increased by only a few (5–15) micrograms daily. Two of the Guernsey calves also recovered completely when given increased amounts of carotene. On the other hand when the remaining two Guernsey calves were treated with Bismo-Pepsol compound, they failed to recover. An autopsy on one of the latter calves revealed a severe fibrinous pleurisy accompanied by a purulent exudate in the pleural cavity. In the more acute areas of inflammation numerous areas of emphysema were present. In the areas with a pneumonia of longer duration there were many abscesses. The intestines were full of food and did not appear to be inflamed although the calf manifested a severe case of diarrhea. It may be significant that at about the same time a number of pure bred calves in the College herd developed a similar type of intestinal and respiratory disorder when only a few days of age. A study of the rations of the dams of these calves indicated that a deficiency of carotene might be responsible. Following these observations a commercial carotene concentrate was administered to all calves during the first few weeks of their lives with favorable results.

The carotene requirements of some of the calves appeared to be slightly higher in cold than in mild or moderate weather. In the case of four Holstein calves and one Guernsey calf maintained at minimum levels of carotene intake, it was observed that these animals showed increased evidence of vitamin A deficiency following a cold spell and that marked improvement followed when the weather again moderated. More marked evidence of this increased requirement might have been observed had the experimental barn not been equipped with a heating and ventilating system which made it possible to maintain a temperature around 50° F. during the winter. The barn temperature never dropped below 28° F. and seldom went above 60° F. except in the summer months.

Observations made on some of the calves indicated the presence of a mild vitamin A deficiency between the time when the whole milk feeding was discontinued and when appreciable amounts of hay were consumed. One calf was supplied with the carotene supplement and another was supplied with fortified cod liver oil during this period. These calves showed some evidence of better health during this period than those that received no supplement. However, no permanent benefit from this supplementation was observed as the calves receiving hay alone seemed to be in about as good condition at the age of four months as those which had received the supplement.

DISCUSSION OF RESULTS

The data obtained in these studies reveal wide variations in the carotene intake necessary to protect different animals against vitamin A deficiency. The requirements of some animals appear to be much higher than others even when consuming similar rations. Some of the animals on timothy hay were apparently protected by a daily intake of 14 micrograms of carotene per pound body weight, whereas others required 19 micrograms. Similar results were obtained with other feedstuffs with the exception that as the biological value of the carotene in a feedstuff increases the range apparently becomes narrower. Similar variations were also observed in the amounts of carotene from different carriers necessary to prevent the symptoms of vitamin A deficiency. These results can be better understood by examining table 1 which summarizes these data. Of the various sources of vitamin A tested the carotene concentrate appears to be the most efficient source as levels of 11 micrograms of carotene protected all animals excepting one Guernsey. The fortified cod liver oil and the alfalfa hay were almost as efficient. With timothy hay as the carrier the requirements were appreciably higher, an intake of 19 micrograms being necessary for all the animals: The carotene of corn silage and of corn meal was utilized as efficiently as that of timothy hay. The requirement for the carotene from alfalfa molasses silage was much higher, a level of 33 micrograms being necessary for complete protection in all animals.

In any attempt to explain the above variations in experimental results, several possibilities suggest themselves. Apparently there is a wide variation in the ability of the animals to utilize the carotene from the same source. This being the case, one would also expect a wide variation in the ability of the animals to use the carotene from different sources. A study of the blood carotene values and a general study of the problem indicates this to be at least part of the explanation. It appears logical to assume that some of the substances in alfalfa silage measured as beta carotene were carotene degradation fractions or other related pigments that have no biological value. The data show that the carotene requirements from these sources were higher than from other carotene carriers. It is also conceivable that part of these pigments may have been carried into the blood and were measured there as carotene.

An unexpected result was obtained with the fortified cod liver oil, in that the requirement appeared to be lower when the oil was fed with little or no roughage than when it was fed with roughage. These results may have been caused by an increased rumination as well as other digestive activity following the feeding of roughage of poor quality. This increased rumination may have resulted in the destruction of part of the vitamin A of the cod liver oil before its passage into the absorptive mechanism of the body.

The data on blood carotene (table 2) indicate that when the serum carotene falls below 0.01 milligram per 100 cc. of serum marked symptoms of vitamin A deficiency are likely to develop at an early date. However, when the serum carotene drops below this value it is difficult to estimate carotene with a satisfactory degree of accuracy. When the value is 0.02 milligram or higher, the animal is usually receiving sufficient carotene for normal growth and well-being. Results obtained in treating intestinal and respiratory diseases indicate that the optimum requirement may be a few micrograms greater than indicated by the appearance of the eye. It also seems that a lowered vitality due to a vitamin A deficiency may have a bearing on the susceptibility of calves to scours and pneumonia. The results, however, show that intakes more than a few micrograms higher than indicated as sufficient by the appearance of the eye are of no further immediate use to the calf so far as growth is concerned. Higher intakes of carotene, on the other hand, may result in greater storage of vitamin A in the body tissues.

A comparison of the data on the two breeds (table 1) indicates that the Guernseys have a slightly higher carotene requirement than the Holsteins, but the difference between the breeds is much less than the differences between individuals within the breeds. In a few instances Holsteins appear to have the higher requirements, but the reverse is more often the case. When a fortified cod liver oil was used as the source of vitamin A, the vitamin A requirements of the Guernseys were no higher than those of the Holsteins.

SUMMARY

The data obtained in this series of studies show that the minimum carotene requirement of growing calves is eleven micrograms per day per pound body weight. This level of carotene intake was sufficient to maintain growth and to prevent the usual vitamin A deficiency symptoms. The adequacy of this amount of carotene, however, depends upon the source of supply of carotene. While it appears desirable that the intake should be considerably above this amount it was surprising to find that increases above that level did not result in marked improvement in growth rate.

The order of availability of carotene to calves from the various sources studied is as follows: 1. Carotene concentrate, 2. Alfalfa hay, 3. Corn silage, 4. Corn meal, 5. Timothy hay, and 6. Alfalfa molasses silage.

ACKNOWLEDGMENTS

Grateful acknowledgment is due: Professor P. S. Williams, for aid in various phases of the investigation. Doctor J. F. Shigley, for looking after the health of the experimental subjects and for performing the post mortem examination. Mr. Oscar H. Struve, chemist at Eastern States Mills, Buffalo, N. Y., for helpful suggestions regarding the determination of carotene.

REFERENCES

(1) BECHDEL, S. I., ECKLES, C. H., AND PALMER, L. S. The vitamin B requirement of the calf. J. DAIRY SC., **9**: 409. 1926.

(2) CONNOR, C. L. Studies on Lipochromes. The quantitative estimation of carotene in blood and tissues. J. Biol. Chem. **77**: 619. 1928.

(3) CONVERSE, H. T., AND MEIGS, E. B. Carotene in the nutrition of dairy calves. Proceedings of 29th annual meeting of A.D.S.A., p. 32. 1934.

(4) CONVERSE, H. T., AND MEIGS, E. B. Carotene and vitamin A in the nutrition of dairy calves. Holstein World, **32**: 79. 1935.
Ibid. Guernsey Br. J., **46**: 590. 1934.

(5) FLORA, C. C., WARD, R. E., BECHDEL, S. I., GUERRANT, N. B., AND DUTCHER, R. A. Carotene in the nutrition of dairy calves. J. DAIRY SC., **22**: 321–328. 1939.

(6) GUILBERT, H. R. Carotene in feeds. J. of Indus. and Eng. Chem. Anal. Edit., **6**: 452. 1934.

(7) GUILBERT, H. R., AND HART, G. H. Minimum vitamin A requirements with particular reference to cattle. J. Nutrit., **10**: 409–427. 1935.

(8) GUILBERT, H. R., MILLER, R. F., AND HUGHES, E. H. The minimum vitamin A and carotene requirement of cattle, sheep, and swine. J. Nutrit., **13**: 543–564. 1937.

(9) HART, G. H., AND GUILBERT, H. R. Vitamin A deficiency as related to reproduction in range cattle. Calif. Exp. Sta., Bull. 560, pp. 1–30. 1933.

(10) JONES, I. R., ECKLES, C. H., AND PALMER, L. S. The role of vitamin A in the nutrition of calves. J. DAIRY SC., **9**: 119. 1926.

(11) KUHLMAN, A. H., GALLUP, W. D., AND WEAVER, E. ''Caratone'' in rations for dairy calves. J. DAIRY SC., **18**: 434. 1935.

(12) MEIGS, E. B. Dairy ration tests show importance of vitamin A in roughage. Yearbook U.S.D.A., pp. 181–185. 1934.

(13) MOORE, L. A. Technique used in studying vitamin A requirements of dairy cattle. J. DAIRY SC., **20**: 427. 1937.

(14) Moore, L. A. Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema, and night blindness in calves. J. Dairy Sc., 21: 114. 1938.

(15) Soldatenkiv, P. F. The influence of vitamin A on the development of calves. Problems Animal Husb. (U.S.S.R.), No. 8, pp. 80–83, 1935. Chem. Abs. 30: 2613⁹.

(16) White, F. D., and Gordon, Ethel M. The estimation of serum carotin. J. Lab. and Clin. Med., 17: 53. 1932.

PENN ST

Alumni

MARCH 1

Volume 26,

E. K. Hibshman '09, *Editor* Alic

THE PENN STATE ALUMNI A
EXECUTIVE BOAR

R. H. Craig '14, *President*
Earl Hewitt '03, 1*st Vice-President*
George M. Arisman '18, 2*nd Vice-President*; Center F
Julia G. Brill '21, 3*rd Vice-President*
C. L. Eshleman '05 Termina
H. I. Smith '07 U. S. Geologi
Miles Horst '14
B. C. Jones '19
J. L. Romig '21
Janet Brownback Gross '31 R. D. 9, Box 114,
W. K. Ulerich '31 Cen

In the News Th

Cover Page—The

• •

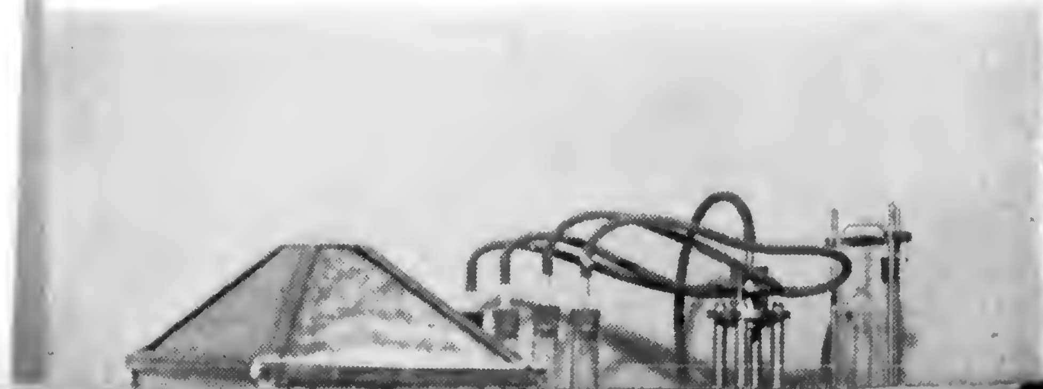
Research Activi

• •

Dr. Frear and Dr. Miller studying factors affe insecticides.

• TWELVE YEARS AGO
News, June, 1928) I at
enumerate some of the m
tant trends in the devel
Agricultural Biochemistry
Pennsylvania State Colleg
sis was placed on the o
personalities of national
national renown who had
in making the department
of its kind in this country
training of agricultural b
is concerned. I described
ence of such outstanding s
Dr. Evan Pugh, Dr. W.
Dr. H. P. Armsby and D
Frear on the development
policies and I concluded w
resume of our own rese
from 1920 to 1928. The
ticle, written at the requ
editor, will deal with se
research activities for t
1928 to 1940.

While there have be
changes in organization a
emphasis, the general pl
prosecution of research w
practically identical with
scribed in 1928. Naturally



tional recognition. Dr. Hunter re-
signed in 1937 to accept a position as
Director of the Nutrition Research
Laboratories of Allied Mills, Inc. He
was succeeded by Dr. R. V. Boucher,
in whose capable hands the work is
progressing rapidly. Dr. Boucher
held a National Research Council
Fellowship at Yale University an

ontemplates, with evident satisfaction,
ing machine for tobacco studies.

(14) MOORE, L. A. Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema, and night blindness in calves. J. DAIRY SC., 21: 114. 1938.

(15) SOLDATENKIV, P. F. The influence of vitamin A on the development of calves. Problems Animal Husb. (U.S.S.R.), No. 8, pp. 80–83, 1935. Chem. Abs. 30: 2613⁹.

(16) WHITE, F. D., AND GORDON, ETHEL M. The estimation of serum carotin. J. Lab. and Clin. Med., 17: 53. 1932.

PENN STATE

Alumni News

MARCH 1940
Volume 26, No. 7

E. K. Hibshman '09, *Editor* Alice I. Thompson, *Associate Editor*

THE PENN STATE ALUMNI ASSOCIATION
EXECUTIVE BOARD

R. H. Craig '14, *President*........222 N. 3rd St., Harrisburg
Earl Hewitt '03, *1st Vice-President*........308 7th Ave., Indiana
George M. Arisman '18, *2nd Vice-President*; Center Rd., School Lane Hills, Lancaster
Julia G. Brill '21, *3rd Vice-President*........Nittany Ave., State College
C. L. Eshleman '05........Terminal Tower Bldg., Cleveland, Ohio
H I. Smith '07........U. S. Geological Survey, Washington, D. C.
Miles Horst '14........6 Maple St., Palmyra
B. C. Jones '19........14 Wood St., Pittsburgh
J. L. Romig '21........R. D., Kennett Square
Janet Brownback Gross '31........R. D. 9, Box 114, South Hills Branch, Pittsburgh
W. K. Ulerich '31........Centre Daily Times, State College

In the News This Month

Cover Page—The Armory

• •

Research Activities

• •

Glee Club Plans Spring Tour

• •

Facing New Problems

• •

Sports

• •

Old Main Columns

• •

District Clubs

• •

Flashes from the Classes

Calendar

APRIL

5	Interfraternity Ball; Varsity Baseball, Princeton at Princeton, N. J.
5-6	Thespian Show
6	Varsity Baseball, Lehigh at Bethlehem; Varsity Lacrosse, Hobart here
7	Dr. Albert W. Beaven, Colgate-Rochester Divinity School, Rochester, New York; Infantry Band Concert, Gullo Conducting
10	Varsity Baseball, Western Maryland here
12	Varsity Baseball, West Virginia here; Varsity Lacrosse, Pennsylvania at Philadelphia
13	Varsity Baseball, West Virginia here; Varsity Lacrosse, Navy at Annapolis, Maryland
14	Dr. Francis P. Miller, Council on Foreign Relations, New York; Glee Club Concert, Gullo Conducting
17	Varsity Baseball, Syracuse here; Varsity Lacrosse, Swarthmore here
19	Freshman Class Dance; Varsity Tennis, Pennsylvania at Philadelphia
20	Engineers Open House; Ag Frolic; Varsity Baseball, Navy here; Varsity Lacrosse, Cornell here; Varsity Tennis, Lehigh at Bethlehem; Varsity Golf, Pittsburgh here
21	Dr. Robert W. Searle, Greater New York Federation of Churches, New York; College Symphony Concert, Fishburn Conducting
23	Varsity Baseball, Temple here

(Continued on page 15)

Issued monthly from September to June inclusive by The Penn State Alumni Association, Inc., at its Lebanon, Pennsylvania, Office of Publication. Subscription price $3.00 per year. Entered as second-class matter at the Post Office, Lebanon, Pa., under Act of March 3, 1879. Communications should be addressed to the office of publication, Box 37, Lebanon, Pa., or Penn State Alumni News, Room 104 Main Building, State College, Pa.

Notice of change in address should be received at the Alumni Office not later than the 15th of the month preceding month of issue.

National Advertising Representative: The Graduate Group, Inc., 30 Rockefeller Plaza, New York.

Dr. Frear and Dr. Miller studying factors affecting "sticking properties" of insecticides.

RESEARCH ACTIVITIES OF THE AGR-BIOCHEMS

By R. Adams Dutcher

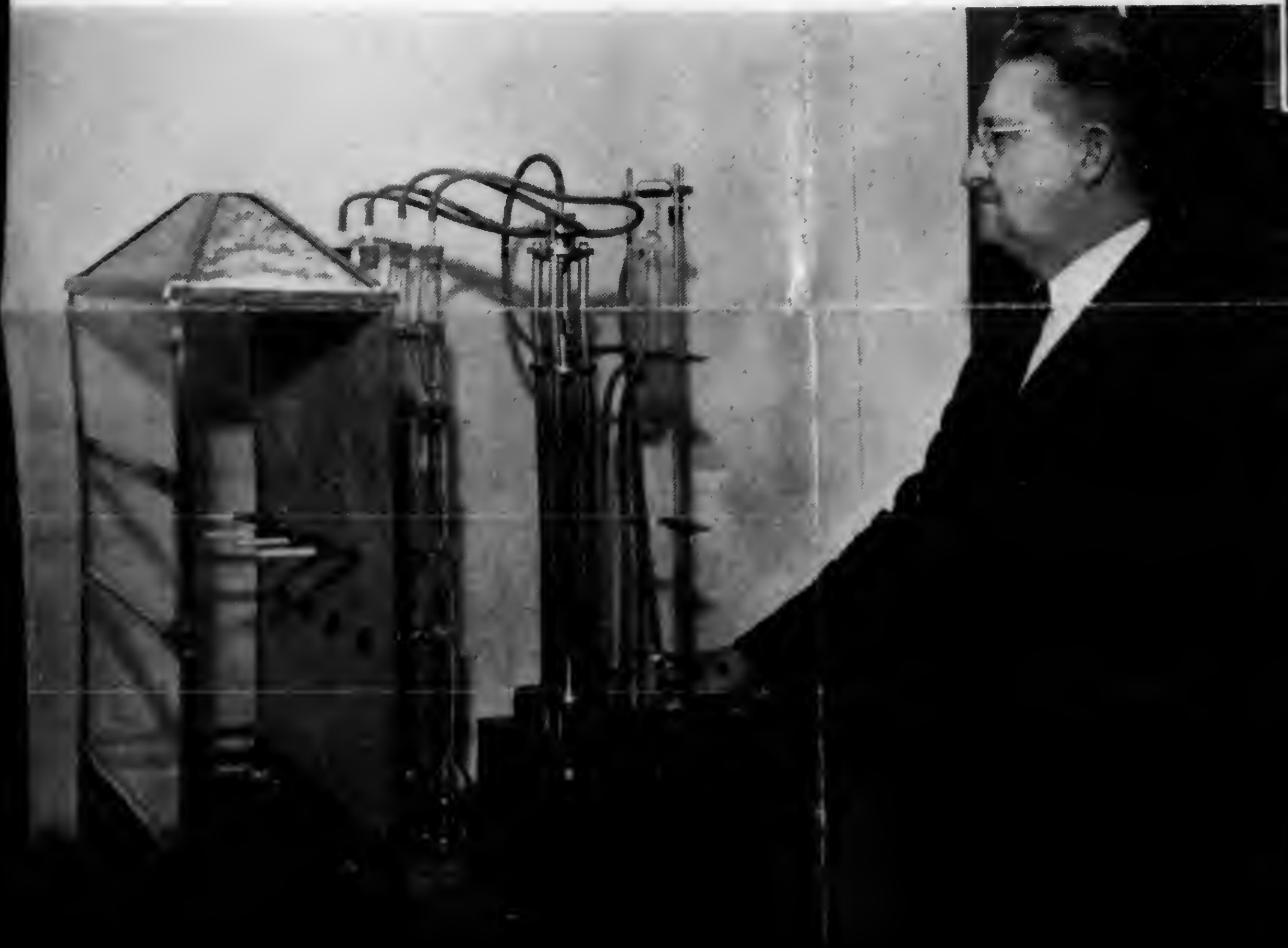
Head of the Department of Agricultural Biochemistry

• TWELVE YEARS AGO (Alumni News, June, 1928) I attempted to enumerate some of the more important trends in the development of Agricultural Biochemistry at The Pennsylvania State College. Emphasis was placed on the outstanding personalities of national and international renown who had contributed in making the department the largest of its kind in this country, so far as training of agricultural biochemists is concerned. I described the influence of such outstanding scientists as Dr. Evan Pugh, Dr. W. H. Jordan, Dr. H. P. Armsby and Dr. William Frear on the development of research policies and I concluded with a brief resume of our own research work from 1920 to 1928. The present article, written at the request of the editor, will deal with some of our research activities for the period 1928 to 1940.

While there have been minor changes in organization and research emphasis, the general plan for the prosecution of research work remains practically identical with that described in 1928. Naturally, there have been changes and additions in personnel during this period. Dr. Hannah E. Honeywell, who had charge of the nutrition laboratory, resigned in 1930 and was succeeded by Dr. N. B. Guerrant, who is recognized as one of the outstanding authorities in the country on questions involving vitamins and their methods of estimation. Owing to his organization ability, we have one of the best laboratories of its kind in this country. We have been honored by being appointed as one of the few referee laboratories, on vitamin assays, by the Federal Government and by the United States Pharmacopoeia.

Dr. R. J. Miller, biochemist in poultry nutrition, resigned in 1929 and was succeeded by Dr. J. E. Hunter, whose cooperative work with Professor Knandel acquired international recognition. Dr. Hunter resigned in 1937 to accept a position as Director of the Nutrition Research Laboratories of Allied Mills, Inc. He was succeeded by Dr. R. V. Boucher, in whose capable hands the work is progressing rapidly. Dr. Boucher held a National Research Council Fellowship at Yale University an

Dr. Knight and Dr. Bechdel removing samples from the cow's storage stomach for assay of vitamin "C".



Dr. Haley contemplates, with evident satisfaction, a new smoking machine for tobacco studies.

came to us from the American Can Company, where he had served as nutrition consultant on foods and food processing problems.

Professor Edward S. Erb, who had charge of our Analytical Service Division for many years, died in February, 1937. He was succeeded by Mr. Arthur L. Haskins ('28), whose experience in industrial laboratories has made him a real asset in developing our policy of cooperative research. Mr. Haskins has also been appointed Referee on Analytical Methods by the Association of Official Agricultural Chemists.

Dr. Walter Thomas, research worker in plant biochemistry, has been transferred to the Department of Horticulture where he is prosecuting studies in plant physiology in cooperation with Dr. Warren B. Mack.

Dr. Russell C. Miller and Dr. Donald E. H. Frear are new additions to the staff. Dr. Miller is employed to conduct cooperative research with the Department of Animal Husbandry and this work, which will be mentioned later, has already attracted considerable attention. Dr. Frear devotes part of his time to cooperative work on horticultural problems with Dr. Anthony and his projects on insecticides, in cooperation with Horticulture, Plant Pathology (Dr. Thurston) and Economic Entomology (Dr. Worthley) are yielding results of practical as well as of scientific value.

Industrial Fellowships

During the past ten years there has been a marked increase in the establishment of subsidized research by various types of industrial organizations. Some of these are in the form of grants to assist professors in initiating new research or to help finance fundamental or practical research already in progress. Most of these, however, are in the form of research fellowships in which the college takes complete responsibility for appointment of research fellows, planning and prosecuting research, etc. Contracts which are drawn by the college specify conditions under which the work is to be done and how research data must be published. Most of the fellowships are designed so that the research fellow works half time on research and half time in graduate work for the doctorate. The following fellowships are now active in the department:

1. *Smith, Kline and French Research Fellowship.*

This is a post-doctorate fellowship which is held by Dr. William Gordon, former National Research Council Fellow at Yale University and recently a member of the Biochemistry staff at Stanford University. Dr. Gordon's present work is a fundamental study of the proteins of milk whey, a by-product of the dairy industry which is often thrown away or fed to domestic livestock.

2. *American Potash Institute Fellowships.*

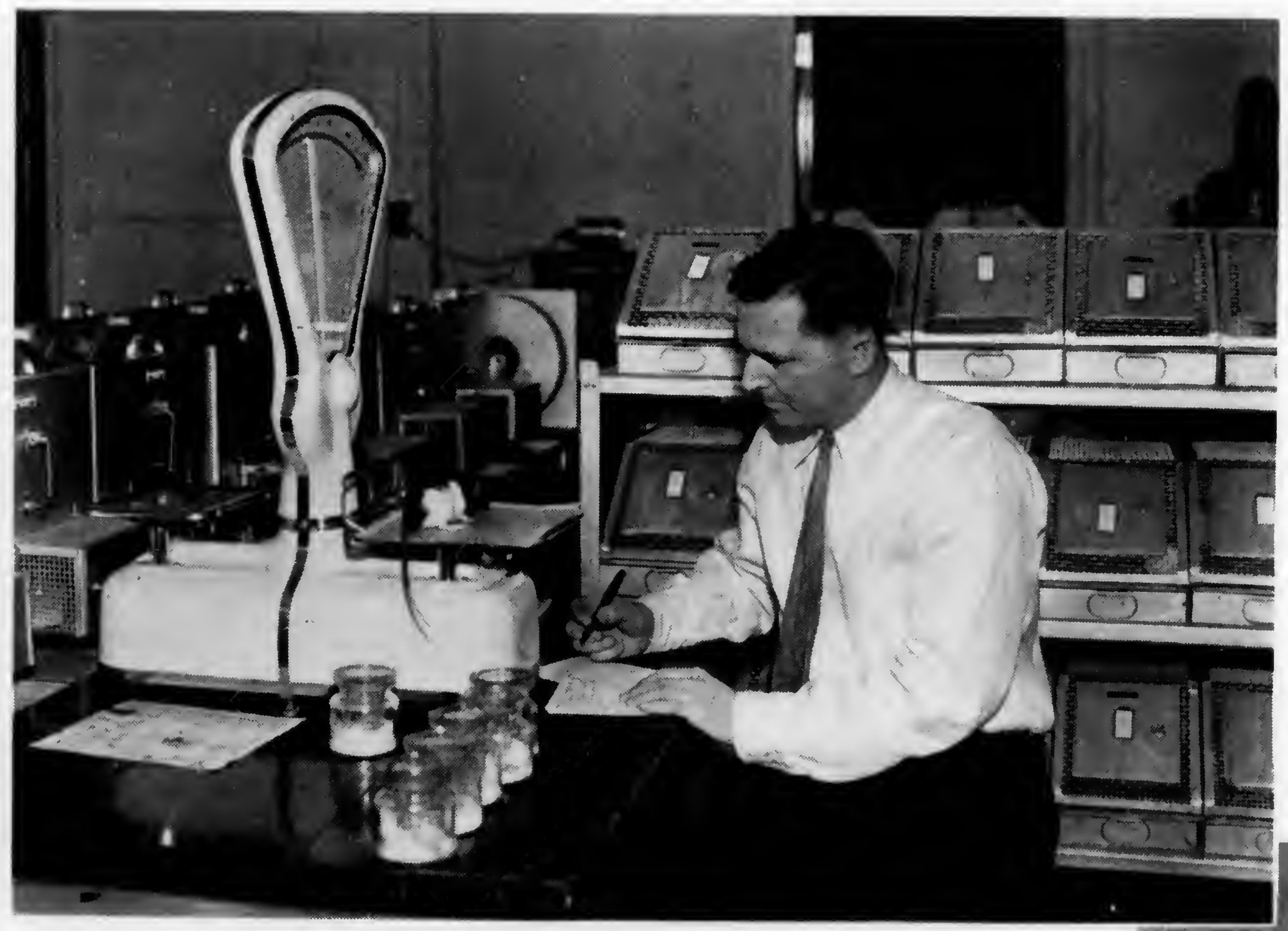
These fellowships are under the direction of Dr. D. E. Haley. Mr. Seymour S. Block and Mr. J. Clyde Underwood hold fellowships under this grant and both of these young men are working for advanced degrees. The principal objective of this work is to study the influence of potassium on all factors affecting quality of cigarleaf tobacco.

3. *Reilly Tar and Chemical Company Fellowship.*

This fellowship is held by Mr. Herman L. King of Michigan State College, who is working for an advanced degree under Dr. Haley. Dr. Haley and Mr. King are assisted in this work by Dr. D. E. H. Frear and Dr. H. N. Worthley. The object of this fellowship is to find new fractions from tar distillates which possess superior insecticidal value but which will have no harmful effect on plants.

4. *Commercial Molasses Corporation Fellowship.*

This fellowship is held by Mr. Walther H. Ott from the Oregon State College, who works under the direction of Dr. R. V. Boucher. This fellowship has as its object a stud



Dr. Guerrant studying effect of exercise on vitamin requirement. Exercise cages in left background.

Dr. Miller examining wool fibers by micro-projection.

the use of [illegible] molasses as an ingredient in poultry feeds with special reference to the importance of the vitamin and mineral content of molasses. Dr. Boucher also assists in the direction of industrial research fellowships which have been established in the Department of Poultry Husbandry.

5. *Castanea Paper Company Fellowship.*

This fellowship is held by Mr. Ralph V. Hurlburt of Montana, who works under the direction of Dr. Haley. The fellowship was established to find, if possible, industrial uses for waste sulfite liquor which has become a stream pollution problem. Excellent progress is being made on this project and it would appear that sulfite liquor residues may be used eventually in the manufacture of plastics and of bases for cheap agricultural fertilizers.

AGRICULTURAL EXPERIMENT STATION PROJECTS

Space will not permit more than a brief statement regarding the regular research program of the department. An entire article could be devoted to the work of Dr. Haley and Dr. Reid (Division of Bacteriology) on factors affecting tobacco quality. With fertilizer experiments in Lancaster County, bacteriological and chemical studies in our laboratories, with fermentation chambers and smoking machines,—these professors and their students are making The Pennsylvania State College known throughout the world as a tobacco research center. Requests for their publications come from all corners of the world.

Our activities in vitamin research have been varied. Cooperative researches with Doctors Bechdel and Dahle of the Dairy Husbandry Department continue to be pleasant and profitable. We have helped Dr. Dahle answer the question "What happens to vitamin A in Cheddar cheese during the ripening or curing process?" We have found that the slight destruction of this vitamin during ripening is offset by moisture losses, which gives the consumer just as many vitamin A units per pound of market cheese as is contained in a pound of unripened or uncured cheese.

Mr. Claude A. Knight, from Alma College, Michigan, is now studying the factors affecting the absorption, metabolism and excretion of vitamin C (ascorbic acid) by the dairy cow. This work is in cooperation with Dr. S. I. Bechdel and Dr. J. F. Shigley. Dr. Shigley has just produced another permanent fistula for us. This gives us another cow "with a window in her stomach."

Under Dr. Guerrant's direction a number of post graduate students have been studying the effect of physical activity on vitamin requirement and the effect of vitamin intake on physiological well-being and desire to exercise. In these studies certain rats are not permitted to exercise at all. Others are allowed to run in revolving cages to their heart's content. These cages automatically record the distance traveled. Other less fortunate rats are placed in revolving cages attached to electric motors. These rats must travel a certain distance every day, regardless of their desires. Dr. Guerrant has proved, what every back-yard gardener knows, that exercise affects intestinal motility. As a rule, fat rats which have not exercised show vitamin A deficiency symptoms earlier than exercised rats and the former have a higher vitamin requirement than the latter. In our studies of vitamin B_1, the reverse is the case, indicating that increased physical activity calls for increased supplies of vitamin B_1. In the first case exercise lowered vitamin A requirement while vitamin B_1, which is necessary for certain types of tissue oxidation, is needed in larger amounts during physical activity. We have also assisted Dr. Bechdel in his work on the vitamin A and D requirements of dairy cattle. Lack of space forbids further discussion of other small animal projects, of which there are many.

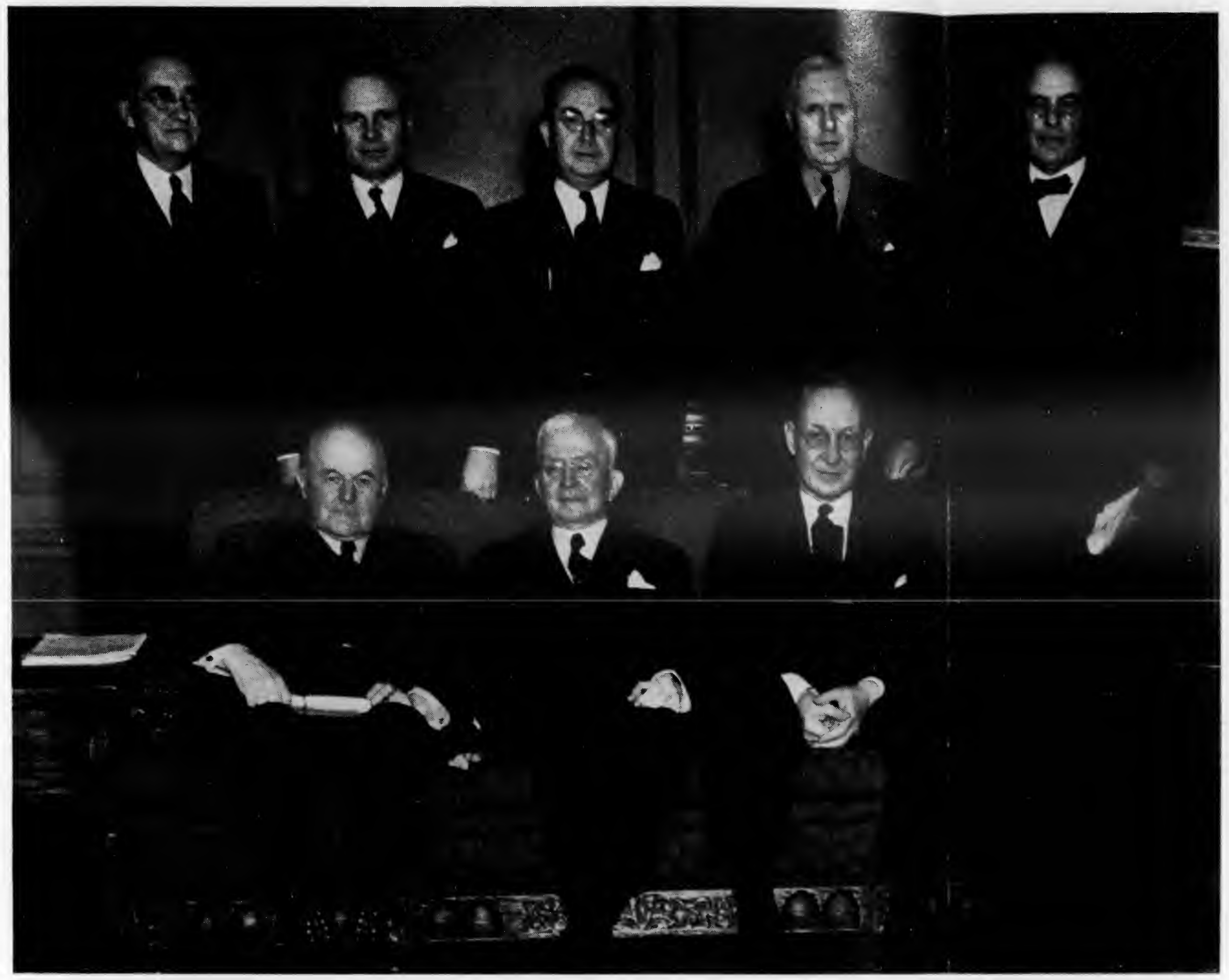
In poultry nutrition, Dr. Boucher is also prosecuting vitamin studies. Doctors Hunter, Murphy and Knandel had established what are now recognized as the accepted vitamin D requirements of growing chicks and laying hens. Dr. Boucher, in cooperation with the Department of Poultry Husbandry, is busy establishing the vitamin D requirement for turkeys. He finds that riboflavin, a new member of the old vitamin B complex has a marked influence on early growth rate and hatchability of eggs. This work is being conducted with chickens and turkeys. Dr. Boucher has also assisted Professor E. W. Callenbach in the game bird project in which a study has been made of the calcium, phosphorus and protein requirements of ring neck pheasants. This project has as its objective the development of a suitable ration for the propagation of ring neck pheasants in captivity.

Dr. H. O. Triebold and his students are studying the organic structure of food fats in relation to fat spoilage, rancidity and keeping quality of fats and fat containing foods. These workers are not only tearing the natural fats to pieces to see what makes them "click" but they are synthesizing artificial fats from pure fatty acids and glycerol. When Dr. Triebold is not teaching, directing fat research or doing committee work,—he is helping the Forestry Department try to find out how the maple tree manages to make such an exquisite concoction of carbohydrates which we insist must accompany our wheat cakes and sausages. Among other activities, Dr. Triebold and his students are studying sources of lead contamination of maple syrup and the chemical composition of natural browse consumed by wild deer. The latter work is in connection with the college program in wild life research.

Dr. R. C. Miller spends his research time cooperating with Professors Bentley, Keith, Ziegler and McCarty of the Department of Animal Husbandry. These researchers, following Dr. Triebold's lead, are also trying to make winter breakfasts more attractive by producing better and cheaper hams and bacon. To this end Dr. Miller is conducting studies on the nutritive requirements of livestock in general, as well as problems involving meat preservation. Farm curing of hams and bacons has been taken out of the realm of uncertainty through the researches of Professors Miller and Ziegler. When time permits, Dr. Miller may be found, with our authority on sheep, Dr. William Henning, gazing through microscopes at wool fibres. This project, on the improvement of wool quality, is comparatively new. Another new project, which has been under way by Doctors Miller and Keith, has as its objective the carotene requirements of swine.

Dr. D. E. H. Frear, in cooperation with Dr. H. N. Worthley of Economic Zoology, applies physical-chemical laws to complex colloidal mixtures of insecticides in order to make arsenic and lead adhere to leaves in lethal amounts for insect control. When this has been accomplished these researchers are forced to find ways and means to place fruits and vegetables on the market sufficiently free of arsenic and lead to pass the State and Government tolerance requirements for these poisons. These workers are also searching for new insecticides to replace those which now contain arsenic and lead. In cooperation with Dr. H. W. Thurston, Jr., Dr. Frear is working on similar studies on copper fungicides, including laboratory investigations on new copper containing materials. With Dr. Anthony, in Horticulture, work is also being conducted on the nutrition of apple trees under controlled conditions.

These are a few of the research activities of the Department of Agricultural Biochemistry. When the new building, Frear Laboratories, is ready for occupancy,—we will have research facilities which will make possible new methods of attack on old problems and the initiation of new research projects for which apparatus and equipment have not been available in the past.



The Executive Committee of the Board of Trustees

Left to right back row: F. A. Heim, John C. Cosgrove '07, James Milholland '11, J. H. M. Andrews '98, E. S. Bayard. Front row: Vance C. McCormick, J. Franklin Shields '92, Dr. Ralph D. Hetzel.

Student Conference

In a new effort to create a permanent organization of student governing bodies in Pennsylvania colleges and universities, the Penn State student government sponsored a conference on the campus on March 1, 2 and 3. Invitations were extended to 23 colleges, and representatives of 17 colleges attended the conference. The delegates numbered approximately 45.

The program included two luncheon and two dinner meetings, together with sectional conferences. President Hetzel spoke at the banquet and gave his emphatic support

(Continued on page 6)

The Varsity Quartet

Glee Club Plans Spring Tour

• The Penn State Glee Club, holders of the Eastern Intercollegiate Championship, will make its annual spring tour during the week of April 8. The first concert will be held in Lancaster on Monday evening, April 8, at the McCaskey High School.

On Tuesday evening, April 9, the club will give a concert in Wilmington, Del., while on Wednesday evening, April 10, they will make their appearance in Philadelphia.

Thursday, April 11, the club will appear in New York City. Fred Waring has invited the Penn State Club to broadcast on the Chesterfield Hour over Station WEAF at 7:00 o'clock in a joint concert with the Waring Glee Club. This is the first time Fred Waring has extended an invitation of this nature to a college or university glee club and it is received as a distinct honor.

Following the broadcast the club will give a concert under the sponsorship of the Penn State Alumni Club of New York City. It is also expected that the Waring Glee Club will join with Penn State in this concert. At 11:00 o'clock Penn State will participate in the West Coast broadcast. These broadcasts will be from the Vanderbilt Theatre at 48th Street, and Penn State alumni in New York are cordially invited to attend. And alumni all over the country are urged to tune in on Penn State. Ed. Lee, Fred Waring's manager, is also an alumnus of Penn State.

While on the tour, the Penn State Glee Club will feature a group of Fred Waring's glee club compositions. In addition there will be special numbers by the Varsity Quartet. Sammy Gallu, president of the club, will sing a number of solos, and Andrew Szejely, talented pianist from Budapest and a member of the freshman class, will give several piano solos.

It will be a great week and the Glee Club is looking forward to it with keen anticipation. For further details concerning the scheduled concerts, alumni should get in touch with the officers of the respective alumni clubs.

In Lancaster—call or write Arthur Boardman, 544 W. Orange St.

In Wilmington, Del.—H. L. Moat, 701 W. 28th St.

In Philadelphia—W. D. Fixter, 236 Rockglen Rd., Penn Wynne.

In New York City—G. L. McGinness, 34-28 209th St., Bayside, L. I.

Student Conference

(Continued from page 5)

to student government as a useful aid to a college president and as a very important educational process in training for democracy.

It was agreed to hold the convention at Penn State next year, with Penn State heading the newly-formed association. Lafayette College gained the vice-presidency and Muhlenberg College was selected as secretary-treasurer. All offices were awarded to the colleges rather than to individuals, with each college required to name the person to fill the office for a one-year term.

Chairman of the Committee on Arrangements was David E. Pergrin '40, president of the senior class.

Robert Hall Craig '14 has been employed as Consulting Engineer by the Borough of State College to make an exhaustive study of sources of an adequate water supply for the State College community.

The Penn State Glee Club

BULLETIN 397 APRIL 1940

SOYBEAN OILMEAL
for Growing Lambs

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

CONTENTS

PAGE

Composition of Rations 2

Experiment 1 3

Experiment 2 5

Experiment 3 6

Experiment 4 7

Experiment 5 9

Experiment 6 10

Composite Study of Experiments 4, 5, and 6 12

Cost of Gains 14

Summary 15

Conclusions 18

Soybean Oilmeal for Growing Lambs*

T. B. KEITH, R. C. MILLER, AND W. L. HENNING†

SOYBEAN oilmeal is an economical source of protein of good quality for livestock feeding. During recent years the production of soybean oilmeal has increased many times, thereby making available a large quantity of this vegetable protein.

The object of the six experiments reported in this bulletin was to determine the optimum percentage of soybean oilmeal that can be used for growing lambs as a source of protein in a concentrate mixture in combination with different proportions of hay.

The outline of the experiments is shown in table 1.

Table 1.—An outline of six experiments in determining the optimum amount of soybean oilmeal for feeding growing lambs.

EXPERIMENT NUMBER	RATIONS COMPARED, IN PAIRS			AVERAGE RATIO, CONCENTRATE TO HAY	NUMBER OF LAMBS
	KIND OF HAY	CONCENTRATE MIXTURE*			
		parts		*parts*	
1	Red clover	Corn 94½	S.B.O.M.† 5	1:1.3	12
	Red clover	Corn 99½		1:1.3	12
2	Timothy-clover	Corn 94	S.B.O.M. 5	1:1.3	11
	Timothy-clover	Corn 99		1:1.3	11
3	Timothy-clover	Corn 94	S.B.O M. 5	1:1.3	15
	Timothy-clover	Corn 89	S.B.O.M. 10	1:1.3	15
4	Red clover	Corn 64	S.B.O.M. 35	3:2	8
	Red clover	Corn 89	S.B.O.M. 10	3:2	8
5	Red clover	Corn 64	S.B.O.M. 35	1:1	11
	Red clover	Corn 89	S.B.O.M. 10	1:1	11
6	Red clover	Corn 64	S.B.O.M. 35	2:3	9
	Red clover	Corn 89	S.B.O.M. 10	2:3	9

*The rations in experiment 1 contain ½ part common salt (sodium chloride); those in experiments 2 and 3 contain ½ part sodium chloride and ½ part finely ground limestone; and those in experiments 4, 5, and 6 contain 1 part sodium chloride.
†S.B.O.M is an abbreviation for soybean oilmeal.

1. A ration composed of red clover hay, and a concentrate mixture of coarsely ground yellow corn 94½ parts, soybean oilmeal 5 parts, and common salt (sodium chloride) ½ part, was compared in experiment 1 with a ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 99½ parts and common salt ½ part. The ratio of concentrate to hay in these feed mixtures was constant between the members of each pair but varied among the pairs. The ratio of concentrate to hay among the pairs ranged from 1:1.2 to 1:1.8.

2. A ration composed of mixed timothy-clover hay and a concentrate mixture of coarsely ground yellow corn 94 parts, soybean oilmeal 5 parts, common salt ½ part, and finely ground limestone ½ part, was compared

---

*Authorized for publication March 30, 1940.

†Assistant Professor of Animal Husbandry, Assistant Professor of Agricultural and Biological Chemistry, and Professor of Animal Husbandry, respectively.

in experiment 2 with a ration composed of mixed timothy-clover hay and a concentrate mixture of coarsely ground yellow corn 99 parts, common salt ½ part, and finely ground limestone ½ part. The ratio of concentrate to hay in these two feed mixtures was constant for the members of each pair but varied among the pairs in the experiment. The ratio of concentrate to hay among the pairs ranged from 1:1 to 1:1.9.

3. A ration composed of mixed timothy-clover hay and a concentrate mixture of coarsely ground yellow corn 94 parts, soybean oilmeal 5 parts, common salt ½ part, and finely ground limestone ½ part, was compared in experiment 3 with a ration composed of mixed timothy-clover hay and a concentrate mixture of corn 89 parts, soybean oilmeal 10 parts, common salt ½ part, and finely ground limestone ½ part. The ratio of concentrate to hay in these two mixtures was constant between the members of each pair, but varied among the pairs. The ratio of concentrate to hay among the pairs ranged from 1:1 to 1:1.8.

4. A ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 64 parts, soybean oilmeal 35 parts, and common salt 1 part, was compared in experiment 4 with a ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 89 parts, soybean oilmeal 10 parts, and common salt 1 part. The ratio of 3 parts concentrate to 2 parts hay in these 2 feed mixtures was constant for all individuals of the experiment.

5. A ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 64 parts, soybean oilmeal 35 parts, and common salt 1 part, was compared in experiment 5 with a ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 89 parts, soybean oilmeal 10 parts, and common salt 1 part. The ratio of 1 part concentrate to 1 part hay in these 2 feed mixtures was constant for all individuals of the experiment.

6. A ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 64 parts, soybean oilmeal 35 parts, and common salt 1 part, was compared in experiment 6 with a ration composed of red clover hay and a concentrate mixture of coarsely ground yellow corn 89 parts, soybean oilmeal 10 parts, and common salt 1 part. The ratio of 2 parts concentrate to 3 parts hay in these 2 rations was constant for all individuals in the experiment.

In all experiments the lambs were fed in matched pairs of the same breed, sex, and similar initial weight. Hampshire, Shropshire, Southdown, Cheviot, and Merino lambs were used, and members of each breed were uniformly distributed throughout each experiment. The daily feed intake was the same for each member of each pair, but varied among the different pairs. The allowance of feed for each member of each pair was based upon the consumption of the member that ate the smaller quantity.

COMPOSITION OF RATIONS

Yellow corn of grade No. 2 was used in all experiments. The soybean oilmeal was made by the expeller process in which the temperature had reached a maximum of 275° to 280° F. The Illini variety composed the greater percentage of the beans used for the meal.

A mixture of red clover hay with a small quantity of timothy, graded as U. S. No. 3, was used in experiment 1. In experiments 2 and 3, a timothy-clover mixture, graded as U. S. No. 1, was used. In experiments 4, 5, and 6, U. S. No. 1 red clover with a small quantity of timothy and alsike was used.

Chemical analyses of the total mixtures as computed from analyses of the ingredients are shown in table 2. The maximum quantity of protein was furnished by the ration composed of soybean oilmeal 35 parts, corn 64 parts, and clover hay, with the concentrate to hay ratio of 3:2. The substitution of soybean oilmeal for 5 per cent of the grain ration did not increase the average protein content of the feed mixture of yellow corn and red clover or timothy hay more than 1 per cent.

In recognition of the relatively low calcium content of timothy hay, 5 per cent of finely ground limestone was included in the rations in experiments 2 and 3. The calcium and phosphorus contents of all rations as reported in table 2 are considered to be sufficient for growing lambs.[1]

Table 2.—Average total chemical composition of each feed mixture in each experiment.

EXPERIMENT NUMBER	FEED MIXTURES, IN PAIRS: KIND OF HAY	CONCENTRATES*				DRY MATTER	PROTEIN	ASH	CALCIUM	PHOSPHORUS
			parts		*parts*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Red clover	Corn	94½	S.B.O.M.	5	86	12.5	5.1	.93	.27
	Red clover	Corn	99½			86	12.0	4.2	.93	.27
2	Timothy-clover	Corn	94	S.B.O.M.	5	84	10.9	4.6	.52	.27
	Timothy-clover	Corn	99			84	10.0	3.4	.44	.26
3	Timothy-clover	Corn	94	S.B.O.M.	5	84	10.9	4.8	.55	.26
	Timothy-clover	Corn	89	S.B.O.M.	10	84	11.7	4.7	.62	.26
4	Red clover	Corn	64	S.B.O.M.	35	87	16.9	4.8	.63	.29
	Red clover	Corn	89	S.B.O.M.	10	87	12.6	4.2	.58	.23
5	Red clover	Corn	64	S.B.O.M.	35	87	16.1	5.0	.75	.26
	Red clover	Corn	89	S.B.O.M.	10	87	11.9	4.5	.72	.21
6	Red clover	Corn	64	S.B.O.M.	35	87	15.1	5.3	.88	.24
	Red clover	Corn	89	S.B.O.M.	10	87	11.9	4.9	.85	.20

*In experiment 1 the concentrate mixture contained ½ part common salt (sodium chloride), in experiments 2 and 3 it contained ½ part common salt and ½ part finely ground limestone, and in experiments 4, 5 and 6, one part common salt.

EXPERIMENT 1

A ration composed of red clover hay and yellow corn was compared in experiment 1 to a ration composed of red clover hay and a concentrate mixture of 94½ parts yellow corn and 5 parts soybean oilmeal, ½ part of common salt being included in each concentrate mixture. The lambs were fed in pairs matched according to breed, sex, and initial weight. Fourteen pairs of lambs were started but only 12 pairs completed the trial. Two pairs of lambs were removed early in the experiment, these lambs being off feed and unthrifty.

As shown by the data in table 3, of the total of 12 pairs of lambs, in 7 pairs the lamb receiving the ration of soybean oilmeal 5 parts, corn 94½ parts, and hay gained in weight more rapidly; in 2 pairs the lamb receiving the ration of corn 99½ parts and hay made the greater gain; and in 3 pairs the gains were equal. The average difference between the average daily gains of the paired lambs was .02 ± .02 pound or 8.7 per cent in favor of the lamb fed soybean oilmeal. According to "Student's" method of determining the statistical significance of differences, the Z-value was 1 ($\frac{.02}{.02} = 1$) and the odds were 291 to 1 for the difference of .02 being significant.

---

[1] Mitchell, H. H. and F. J. McClure. Mineral Nutrition of Farm Animals. Nat. Res. Coun. Bull. 99, p. 67.

In 156 comparisons of weekly gains in body weight of the pairs, 74 favored the lamb which received the ration of soybean oilmeal 5 parts, corn 94½ parts, and hay; 28 showed no difference; and 54 favored the lamb fed no soybean oilmeal. The ideal result, if chance alone operated, would be 78 comparisons favoring either the lamb receiving the ration of soybean oilmeal 5 parts, corn 94½ parts and hay, or the lamb receiving the corn and hay ration without soybean oilmeal. The deviation from the ideal is 1.6 times the standard deviation ($\sqrt{.5 \text{ x } .5 \text{ x } 156} = 6.2$) and is not considered to be highly significant.

Table 3.—Live weights, gains, and feed economy of 12 pairs of lambs fed corn and red clover hay, with 1 member of each pair having 5 parts (per cent) of its corn replaced by soybean oilmeal; feeding period 91 days; experiment 1.

PAIRS	AGE AT BEGINNING	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN	RATIO CONCENTRATES TO HAY
	days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*1 to*
No. 1								
5 parts S.B.O.M.	163	57	80	23	.25	2.3	914	1.2
All corn	113	57	83	26	.29	2.3	810	1.2
No. 2								
5 parts S.B.O.M.	137	52	81	29	.32	2.4	738	1.3
All corn	111	49	76	27	.30	2.3	786	1.3
No. 3								
5 parts S.B.O.M	106	41	68	27	.30	2.0	669	1.2
All corn	158	40	65	25	.27	2.0	770	1.2
No. 4								
5 parts S.B.O.M.	112	55	79	24	.26	2.1	802	1.2
All corn	166	52	73	21	.23	2.1	914	1.2
No. 5								
5 parts S.B.O.M.	156	47	73	26	.29	2.2	780	1.2
All corn	128	43	74	26	.29	2.2	780	1.2
No. 6								
5 parts S.B.O.M.	128	40	57	17	.19	1.3	701	1.3
All corn	129	41	54	13	.14	1.3	917	1.3
No. 7								
5 parts S.B.O.M.	135	47	69	22	.24	1.8	728	1.2
All corn	133	45	62	17	.19	1.7	935	1.2
No. 8								
5 parts S.B.O.M.	133	42	64	22	.24	1.6	674	1.3
All corn	119	43	60	17	.19	1.6	871	1.3
No. 9								
5 parts S.B.O.M.	153	51	67	16	.18	1.7	859	1.4
All corn	134	52	71	19	.21	1.7	799	1.4
No. 10								
5 parts S.B.O.M.		58	82	24	.26	2.1	802	1.8
All corn	147	59	81	22	.24	2.1	876	1.8
No. 11								
5 parts S.B.O.M.	134	52	73	21	.23	2.1	895	1.3
All corn	135	54	75	21	.23	2.1	896	1.3
No. 12								
5 parts S.B.O.M.	136	57	78	21	.23	2.1	911	1.3
All corn	137	63	84	21	.23	2.1	910	1.3
Average								
5 parts S.B.O.M.	136	50	73	23	.25	1.98	789	1.3
All corn	134	50	72	21	.23	1.97	855	1.3

The soybean oilmeal lambs required 66 pounds or 8.4 per cent less feed for each 100 pounds of gain than the lambs fed the corn and hay ration without soybean oilmeal. The lambs fed soybean oilmeal refused an average of 3.5 pounds of feed per head for the entire experiment as compared to 8.8 pounds per head for the lambs fed corn and clover hay without soybean oilmeal.

EXPERIMENT 2

A ration composed of a concentrate mixture of soybean oilmeal 5 parts and corn 94 parts, plus hay was compared in experiment 2 with a ration composed of a concentrate mixture of corn 99 parts plus hay, each concentrate mixture containing ½ part common salt and ½ part finely ground limestone. In this experiment the hay was approximately equal parts by weight timothy and red clover. The ratio of grain to hay varied among the pairs and ranged from 1:1 to 1:1.9. The data relating to gains and feed economy of the lambs fed in experiment 2 are shown in table 4. The pairs of lambs receiving the ration with a low ratio of concentrate to hay made lower gains than the lambs fed approximately equal weights of concentrate and hay. The lambs fed the ration of soybean oilmeal 5 parts, corn 94 parts, and hay averaged .01 pound higher in daily gain than the lambs fed the corn and hay without soybean oilmeal. This difference is not statistically significant. The lambs fed the ration of soybean oilmeal 5 parts, corn 94 parts, and hay required an average of 7 per cent less feed for each 100 pounds of gain than those lambs fed corn and hay without soybean oilmeal.

Of the 11 pairs of lambs, in 5 pairs the lamb receiving the ration of soybean oilmeal 5 parts, corn 94 parts, and hay gained in weight more rapidly, in 5 pairs the lamb receiving the ration of corn and hay without soybean oilmeal gained more rapidly, and in 1 pair the gains were equal.

In 166 comparisons of weekly gains in body weight of the pairs, 60 favored the lamb which received the ration of soybean oilmeal 5 parts, corn 94 parts, and hay; 43 were neutral; and 63 favored the lamb fed the ration of corn and hay without soybean oilmeal.

In this experiment, the replacing of 5 per cent of the corn with soybean oilmeal did not increase the gains of lambs when fed a timothy-clover hay mixture.

Table 4.—Live weights, gains, and feed economy of 11 pairs of lambs fed corn and timothy-red clover hay, 1 member of each pair having 5 parts (per cent) of its corn replaced by soybean oilmeal; experiment 2.

PAIRS	AGE AT BEGINNING	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN	RATIO CONCENTRATES TO HAY
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	1 *to*
No. 1									
5 parts S.B.O.M.	174	112	52	69	17	.15	1.87	1232	1.1
All corn	173	112	52	70	18	.16	1.86	1155	1.1
No. 2									
5 parts S.B.O.M.	197	112	66	87	21	.19	1.86	993	1.1
All corn	192	112	62	84	22	.20	1.86	949	1.1
No. 3									
5 parts S.B.O.M	142	112	60	78	18	.16	1.86	1160	1.1
All corn	155	112	59	80	21	.19	1.86	994	1.1
No. 4									
5 parts S.B.O.M.	180	112	68	99	31	.28	2.14	775	1.1
All corn	156	112	69	95	26	.23	2.14	921	1.1
No. 5									
5 parts S.B.O.M	192	112	52	68	16	.14	1.60	1120	1.0
All corn	163	112	53	71	18	.16	1.59	987	1.0
No. 6									
5 parts S.B.O.M.	127	112	44	66	22	.20	1.75	893	1.1
All corn	173	112	43	62	19	.17	1.75	1038	1.1
No. 7									
5 parts S.B.O.M	187	112	56	68	12	.11	1.64	1531	1.3
All corn	186	112	60	71	11	.10	1.65	1684	1.3
No. 8									
5 parts S.B.O.M.	132	105	45	53	8	.08	1.17	1640	1.4
All corn	133	105	44	54	10	.10	1.18	1318	1.4
No. 9									
5 parts S.B.O.M.		91	42	54	12	.13	.86	652	1.9
All corn		91	40	45	5	.05	.85	1554	1.9
No. 10									
5 parts S.B.O.M.	134	105	43	55	12	.12	1.36	1188	1.6
All corn	145	105	44	56	12	.12	1.36	1186	1.6
No. 11									
5 parts S.B.O.M.	149	105	44	62	18	.17	1.64	959	1.2
All corn		105	45	59	14	.13	1.65	1236	1.2
Average									
5 parts S.B.O.M.	175	108	52	69	17	.16	1.61	1100	1.3
All corn	148	108	52	68	16	.15	1.61	1184	1.3

EXPERIMENT 3

In experiment 3, a concentrate mixture composed of soybean oilmeal 5 parts, and corn 94 parts plus mixed timothy-clover hay was compared with a concentrate mixture composed of soybean oilmeal 10 parts, and corn 89 parts plus mixed timothy-clover hay. Each concentrate mixture contained ½ part common salt and ½ part finely ground limestone.

Experiment 3 was conducted concurrently with experiment 2. The data on the performance of the individuals are shown in table 5. The pairs of lambs receiving the ration with the highest ratio of concentrate to hay made a higher average daily gain than those lambs receiving the ration with the low ratio of concentrate to hay. The average daily gains of the lambs fed the two rations were the same and the feed requirement for each 100 pounds gain was approximately the same.

Table 5.—Live weights, gains, and feed economy of 15 pairs of lambs fed corn and timothy-clover hay, 1 member of each pair having 5 parts (per cent) and the other 10 parts (per cent) of its corn replaced by soybean oilmeal; experiment 3.

PAIRS	AGE AT BEGINNING	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN	RATIO CONCENTRATES TO HAY
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	1 *to*
No. 1									
5 parts S.B.O.M.	159	112	43	64	21	.19	1.72	920	1.2
10 parts S.B.O.M.	159	112	41	70	29	.26	1.71	660	1.2
No. 2									
5 parts S.B.O.M.	118	112	51	77	26	.23	1.98	852	1.1
10 parts S.B.O.M.	137	112	49	83	34	.30	1.98	652	1.1
No. 3									
5 parts S.B.O.M	113	112	56	69	13	.12	1.65	1425	1.4
10 parts, S.B.O.M	146	112	60	72	12	.11	1.68	1568	1.4
No. 4									
5 parts S.B.O.M.	117	112	69	104	35	.31	2.17	694	1.1
10 parts S.B.O.M.	158	112	74	100	26	.23	2.16	930	1.1
No. 5									
5 parts S.B.O.M.	117	112	54	78	24	.21	2.06	960	1.0
10 parts S.B.O.M.	146	112	63	84	21	.19	2.04	1088	1.0
No. 6									
5 parts S.B.O.M.	171	98	63	87	24	.24	2.21	900	1.2
10 parts S.B.O.M.	169	98	67	88	21	.21	2.21	1033	1.2
No. 7									
5 parts S.B.O.M.	175	112	49	70	21	.19	1.76	939	1.2
10 parts S.B.O.M.	160	112	56	76	20	.18	1.75	980	1.2
No. 8									
5 parts S.B.O.M.	128	105	36	49	13	.12	1.06	861	1.3
10 parts S.B.O.M,	128	105	37	48	11	.10	1.05	1005	1.3
No. 9									
5 parts S.B.O.M.	140	105	51	60	9	.09	1.16	1351	1.4
10 parts S.B.O.M.	136	105	52	59	7	.07	1.16	1746	1.4
No. 10									
5 parts S.B.O.M.	128	105	48	64	16	.15	1.64	1074	1.2
10 parts S.B.O.M.	132	105	49	68	19	.18	1.61	1613	1.2
No. 11									
5 parts S.B.O.M.	151	105	47	64	17	.16	1.55	958	1.3
10 parts S.B.O.M.	149	105	49	66	17	.16	1.55	958	1.3
No. 12									
5 parts S.B.O.M.	152	105	56	66	10	.10	1.42	1489	1.2
10 parts S.B.O.M.	153	105	56	72	16	.15	1.42	931	1.2
No. 13									
5 parts S.B.O.M.	149	105	60	74	14	.13	1.66	1245	1.3
10 parts S.B.O.M.	145	105	62	78	16	.15	1.66	1091	1.3
No. 14									
5 parts S.B.O.M.	145	105	52	67	15	.14	1.64	1149	1.4
10 parts S.B.O.M.		105	54	67	13	.12	1.63	1318	1.4
No. 15									
5 parts S.B.O.M.	148	105	52	62	10	.10	1.24	1308	1.8
10 parts S.B.O.M.		105	54	66	12	.12	1.24	1088	1.8
Average									
5 parts S.B.O.M.	141	107	52	70	18	.17	1.66	1075	1.3
10 parts S.B.O.M.	148	107	55	73	18	.17	1.66	1111	1.3

EXPERIMENT 4

In experiment 4, a concentrate mixture composed of soybean oilmeal 35 parts and corn 64 parts, was compared with a concentrate mixture composed of soybean oilmeal 10 parts, and corn 89 parts. Red clover hay was fed as roughage in both rations, and the concentrate mixture of each contained 1 part of common salt. The concentrate to hay ratio, 3:2, was the same among pairs.

The data for gains and economy of gains are shown in table 6. All lambs fed each ration made high average daily gains. Individual gains ranged from .15 to .41 pound. The lambs receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay averaged .03 pound or 11 per cent more gain daily than the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The odds that this difference is significant were 59 to 1. The lambs fed the ration of soybean oilmeal 35 parts, corn 89 parts, and hay required an average of 6 per cent less feed for each hundred pounds of gain than the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. This is a significant difference.

Of the total of 8 pairs of lambs, in 6 pairs the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay gained more rapidly, in 1 pair the lamb receiving the ration of soybean oilmeal 10 parts, corn 89 parts, and hay made the greater gain, and in 1 pair the gains were equal.

In 93 comparisons of weekly gains in body weight of the pairs, 41 favored the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay; 17 were the same; and 35 favored the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The ideal result, if chance alone operated, would be 46.5 comparisons in favor of either the lamb fed 35 parts or the lamb fed 10 parts of soybean oilmeal in its concentrate mixture. The deviation of 3 from this ideal is equal to approximately .6 of the standard deviation ($\sqrt{.5 \times .5 \times 93} = 4.8$).

Table 6.—Live weights, gains, and feed economy of 8 pairs of lambs fed corn and red clover hay, 1 member of each pair having 35 parts (per cent) and the other member 10 parts (per cent) of its corn replaced by soybean oilmeal; ratio of concentrate to hay 3:2; experiment 4.

PAIRS	AGE AT BEGINNING	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
No. 1								
35 parts S.B.O.M.	144	94	43	67	24	.25	1.59	624
10 parts S.B.O.M.	141	94	45	67	22	.23	1.59	681
No. 2								
35 parts S.B.O.M.	175	78	64	93	29	.37	2.31	621
10 parts S.B.O.M.	139	78	61	88	27	.35	2.31	668
No. 3								
35 parts S.B.O.M.	156	78	57	83	26	.33	2.03	610
10 parts S.B.O.M.	179	78	61	84	23	.29	2.03	688
No. 4								
35 parts S.B.O.M.	177	78	56	83	27	.35	2.12	614
10 parts S.B.O.M.	174	78	56	79	23	.29	2.13	719
No. 5								
35 parts S.B.O.M.		94	47	61	14	.15	1.25	838
10 parts S.B.O.M.		94	49	63	14	.15	1.25	836
No. 6								
35 parts S.B.O.M.	172	78	62	87	25	.32	1.99	622
10 parts S.B.O.M.	170	78	61	81	20	.26	1.99	776
No. 7								
35 parts S.B.O.M.	169	42	47	64	17	.41	1.95	482
10 parts S.B.O.M.	169	42	49	63	14	.33	1.96	587
No. 8								
35 parts S.B.O.M.	166	94	48	77	29	.31	2.04	662
10 parts S.B.O.M.	169	94	51	81	30	.32	2.04	641
Average								
35 parts S.B.O.M.	166	80	53	77	24	.31	1.91	659
10 parts S.B.O.M.	163	80	54	76	22	.28	1.91	700

EXPERIMENT 5

A comparison was made in experiment 5 of a concentrate mixture composed of soybean oilmeal 35 parts and corn 64 parts with red clover hay as roughage and a concentrate mixture composed of soybean oilmeal 10 parts and corn 89 parts also with red clover hay. Each concentrate mixture contained 1 part of common salt. This experiment was conducted concurrently with experiment 4, the ratio of grain to hay being 1:1 instead of 3:2 as in experiment 4.

Data for gains and feed economy of the individual lambs of experiment 5 are shown in table 7. The lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay gained an average of .02 pound daily or 9 per cent more than the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. Ten per cent more feed was required for each 100 pounds gain by the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay than by the lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay. Of the 11 pairs of lambs, in 8 pairs the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay gained more rapidly, in 2 pairs the lamb receiving the ration of soybean oilmeal 10 parts, corn 89 parts, and hay made the greater gain, and in 1 pair the gains were equal.

When the data are analyzed by "Student's" method, the standard deviation of the difference is found to be .02 and the odds for this difference being significant are 84 to 1.

In 148 comparisons of weekly gains in body weight of the pairs, 62 favored the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay, 44 favored the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay, and 42 showed no difference. The ideal result, if chance alone operated, would be 74 comparisons favoring either the lamb fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay or the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The deviation of 9 from this ideal is equal to approximately 1.5 standard deviations ($\sqrt{.5 \times .5 \times 148} = 6.1$).

Table 7.—Live weights, gains and feed economy of 11 pairs of lambs fed corn and red clover hay, 1 member of each pair having 35 parts (per cent) and the other member 10 parts (per cent) of its corn replaced by soybean oilmeal; ratio of concentrate to hay 1:1; experiment 5.

PAIRS	AGE AT BEGINNING	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
No. 1								
35 parts S.B.O.M.	155	94	49	78	29	.31	2.13	690
10 parts S.B.O.M.		94	51	77	26	.28	2.13	770
No. 2								
35 parts S.B.O.M.	169	94	52	73	21	.22	1.70	760
10 parts S.B.O.M.	163	94	54	71	17	.19	1.70	939
No. 3								
35 parts S.B.O.M.	167	57	72	90	18	.32	2.34	739
10 parts S.B.O.M.	177	57	63	78	15	.27	2.29	871
No. 4								
35 parts S.B.O.M.	165	94	46	64	18	.19	1.58	823
10 parts S.B.O.M.	160	94	47	62	15	.16	1.58	988
No. 5								
35 parts S.B.O.M.	165	94	46	69	23	.25	1.71	697
10 parts S.B.O.M.	126	94	50	73	23	.25	1.71	697
No. 6								
35 parts S.B.O.M.	158	94	48	72	24	.25	1.96	767
10 parts S.B.O.M.		94	47	74	27	.29	1.96	683
No. 7								
35 parts S.B.O.M.	158	94	51	79	28	.30	2.09	702
10 parts S.B.O.M.	165	94	51	76	25	.27	2.09	786
No. 8								
35 parts S.B.O.M.	148	94	42	54	12	.13	1.25	975
10 parts S.B.O.M.	133	94	42	55	13	.14	1.25	906
No. 9								
35 parts S.B.O.M.	144	94	42	64	22	.23	1.61	689
10 parts S.B.O.M.	146	94	42	60	18	.19	1.61	843
No. 10								
35 parts S.B.O.M.	158	94	44	63	19	.20	1.47	725
10 parts S.B.O.M.		94	45	61	16	.17	1.46	860
No. 11								
35 parts S.B.O.M.	140	94	46	67	21	.22	1.59	711
10 parts S.B.O.M.	140	94	54	73	19	.20	1.59	786
Average								
35 parts S.B.O.M.	157	91	49	70	21	.24	1.77	753
10 parts S.B.O.M.	151	91	50	69	20	.22	1.76	830

EXPERIMENT 6

A comparison was made in experiment 6 of a ration composed of a concentrate mixture of soybean oilmeal 35 parts, and corn 64 parts, plus red clover hay and a ration composed of a concentrate mixture of soybean oilmeal 10 parts, and corn 89 parts to which red clover hay also was added. Each concentrate mixture contained 1 part common salt. This experiment was conducted concurrently with experiments 4 and 5. The ratio of concentrate to hay was 2:3 for the 8 pairs.

Data relating to weights, gains and economy of gains of the lambs of experiment 6 are shown in table 8. Eleven pairs began this experiment, and nine completed the trial. Two lambs died during the test; consequently, their mates were removed from the experiment. The deaths of the lambs followed the development of an unthrifty condition which developed previous to the beginning of the experiment.

Table 8.—Live weights, gains, and feed economy of 11 pairs of lambs fed corn and red clover hay, 1 member of each pair having 35 parts (per cent) and the other member 10 parts (per cent) of its corn replaced by soybean oilmeal; ratio of concentrate to hay 2:3; experiment 6.

PAIRS	AGE AT BEGINNING	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
No. 1								
35 parts S.B.O.M.	146	94	43	63	20	.21	1.72	809
10 parts S.B.O.M.	146	94	44	60	17	.17	1.72	1011
No. 2								
35 parts S.B.O.M.		77	49	55	6	.08	1.21	1555
10 parts S.B.O.M.		77	49	57	8	.10	1.21	1164
No. 3								
35 parts S.B.O.M.	144	94	45	61	16	.17	1.35	794
10 parts S.B.O.M.	150	94	44	53	9	.09	1.35	1415
No. 4								
35 parts S.B.O.M.	142	94	38	53	15	.16	1.22	764
10 parts S.B.O.M.	144	94	38	50	12	.13	1.22	954
No. 5								
35 parts S.B.O.M.	140	94	49	57	8	.08	1.24	1451
10 parts S.B.O.M.	141	94	48	57	9	.09	1.24	1302
No. 6								
35 parts S.B.O.M.	144	94	54	70	16	.17	1.46	858
10 parts S.B.O.M.	143	94	54	67	13	.14	1.47	1060
No. 7								
35 parts S.B.O.M.	140	94	57	68	11	.12	1.40	1199
10 parts S.B.O.M.	148	94	51	60	9	.09	1.40	1466
No. 8								
35 parts S.B.O.M.	140	94	46	73	27	.29	1.86	648
10 parts S.B.O.M.	140	94	48	73	25	.27	1.86	700
No. 9								
35 parts S.B.O.M.	126	94	57	69	12	.13	1.57	1226
10 parts S.B.O.M.	165	94	53	59	6	.06	1.57	2452
Average								
35 parts S.B.O.M.	140	92	49	63	15	.16	1.45	1034
10 parts S.B.O.M.	147	92	48	60	12	.13	1.45	1280

The lambs in this experiment did not gain as rapidly as those of experiments 4 and 5. Those fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay made an average daily gain which was .03 pound or 23 per cent more than the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The standard deviation of this difference is .05 and the odds for this difference being significant are 18 to 1. The feed requirement per unit of gain was 19 per cent less for the lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay than for the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay.

Of the total of 9 pairs of lambs, in 7 pairs the lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay gained more rapidly, and in 2 pairs the lambs receiving the ration of soybean oilmeal 10 parts, corn 89 parts, and hay made the greater gain.

In 123 comparisons of weekly gains in body weight of the pairs, 56 favored the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay; 43 favored the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay; and 24 comparisons showed no difference. The ideal result, if chance alone was the determining factor, would be 61.5 comparisons favoring either the lamb fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay or the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The deviation of 6.5 from this ideal is equal to approximately 1.2 standard deviations ($\sqrt{.5 \text{ x } .5 \text{ x } 123} = 5.5$).

COMPOSITE STUDY OF EXPERIMENTS 4, 5, AND 6

The data presented in table 9 show an increase in the average daily gain of .03 pound or 15 per cent, and a saving of 94 pounds or 13 per cent of feed for each 100 pounds gain, in the lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay over those fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay.

The standard deviation of the difference, .03 pound, is .02 pound. When the data are analyzed by "Student's" method a Z-value of 1.5 is obtained which with 28 individuals gives odds exceeding 10,000 to 1 that the difference is significant. While a difference of .03 pound is not large, it is of importance in the case of lambs, especially so in the light of the small standard deviation which attests the consistent differences existing between pair mates. The data indicate, therefore, that the ration of soybean oilmeal 35 parts, corn 64 parts, and red clover hay was of greater value for growth as measured by gain in liveweight than was the ration of soybean oilmeal 10 parts, corn 89 parts, and red clover hay.

Of the total of 28 pairs of lambs, in 21 pairs the lamb receiving the ration of soybean oilmeal 35 parts, corn 64 parts, and hay gained more rapidly; in only 5 pairs the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay made the greater gain; and in two pairs the gains were the same.

In 364 comparisons of weekly gains in body weight, 159 favored the lamb fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay; 83 showed no difference; and 122 favored the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The ideal result, if chance alone operated, would be 182 comparisons favoring either the lamb fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay or the lamb fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay. The deviation of 18.5 from this ideal is equal to approximately 1.9 standard deviations ($\sqrt{.5 \text{ x } .5 \text{ x } 364} = 9.5$).

Table 9.—A comparison of the average gains and feed requirements of lambs fed 35 parts (per cent) and 10 parts (per cent) soybean oilmeal concentrate rations with red clover hay.

RATION	NUMBER INDIVIDUALS	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
35 parts S.B.O.M.	28	50	70	.23	1.68	744
10 parts S.B.O.M.	28	50	68	.20	1.68	838

As shown in table 10 the lambs fed the ration with 3 parts concentrate and 2 parts hay made the most rapid and economical gains. The lambs fed the high concentrate ration, ratio 3:2, made .06 pound or 26 per cent greater average daily gain and required 124 pounds or 16 per cent less total feed for each 100 pounds gain than the lambs fed the ration of 1 part concentrate and 1 part hay. The lambs fed the ration with the concentrate to hay ratio of 3:2 made .15 pound or 107 per cent higher average daily gain and required 490 pounds or 42 per cent less total feed for each 100 pounds gain than the lambs fed the ration of 2 parts concentrate and 3 parts hay. The lambs fed the ration of equal parts concentrate and hay made .09 pound or 64 per cent higher average daily gain and required 366 pounds or 32 per cent less total feed for each 100 pounds gain than the lambs fed the ration with the concentrate to hay ratio of 2:3.

The lambs fed the ration of 3 parts concentrate and 2 parts hay consumed .14 pound or 8 per cent more total daily feed than the lambs fed the ration of 1 part concentrate and 1 part hay, and .46 pound or 32 per cent more total daily feed than the lambs fed the ration of 2 parts concentrate and 3 parts hay. The lambs fed the ration of equal parts concentrate and hay consumed .32 pound or 22 per cent more total daily feed than the lambs fed 2 parts concentrate and 3 parts hay.

Table 10.—A comparison of the average gains, and average feed intake and feed requirements of lambs fed rations with three different ratios of concentrate to hay.

RATIO OF CONCENTRATE TO HAY	NUMBER OF INDIVIDUALS	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 POUNDS GAIN
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
3:2	16	53.6	76.3	22.8	.29	1.91	667
1:1	22	49.3	69.7	20.4	.23	1.77	791
2:3	18	48.2	61.6	13.2	.14	1.45	1157

The total quantity of feed refused by all lambs and the number of feed refusals by the lambs in experiments 4, 5, and 6 are shown in table 11. The number of feed refusals of experiments 1, 2, and 3 were not recorded. The total quantity of feed refused and the

Table 11.—Total feed refused and number of feed refusals by lambs in experiments 4, 5, and 6.

EXPERIMENT	RATION	NUMBER OF INDIVIDUALS	RATIO CONCENTRATE TO HAY	FEED REFUSED	NUMBER OF REFUSALS
				pounds	
1	5 parts S.B.O.M.	12	1:1.3	42.2	
	All corn	12	1:1.3	105.3	
2	5 parts S.B.O.M	11	1:1.3	37.7	
	All corn	11	1:1.3	38.8	
3	5 parts S.B.O.M.	15	1:1.3	80.3	
	10 parts S.B.O.M.	15	1:1.3	65.2	
4	35 parts S.B.O.M.	8	3:2	8.9	271
	10 parts S.B.O.M.	8	3:2	11.8	394
5	35 parts S.B.O.M.	11	1:1	12.8	1609
	10 parts S.B.O.M.	11	1:1	10.8	644
6	35 parts S.B.O.M.	9	2:3	8.2	330
	10 parts S.B.O.M.	9	2:3	10.7	601

number of refusals were the greatest for the members of the pairs fed the smaller quantity of soybean oilmeal in experiments 4 and 6, and the greatest for the members of the pairs fed the larger quantity of soybean oilmeal in experiment 5.

COST OF GAINS

Nomographs are presented on pages 16 and 17 with which the lamb feeder may determine the cost of 100 pounds of gain with prevailing prices of concentrate and hay.

Nomograph 1 presents the cost of 100 pounds of gain of lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay, the ratios of concentrate to hay being 3:2, 1:1, and 2:3, respectively. Nomograph 2 presents the cost of 100 pounds of gain of lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay, the ratios of concentrate to hay being 3:2, 1:1, and 2:3, respectively.

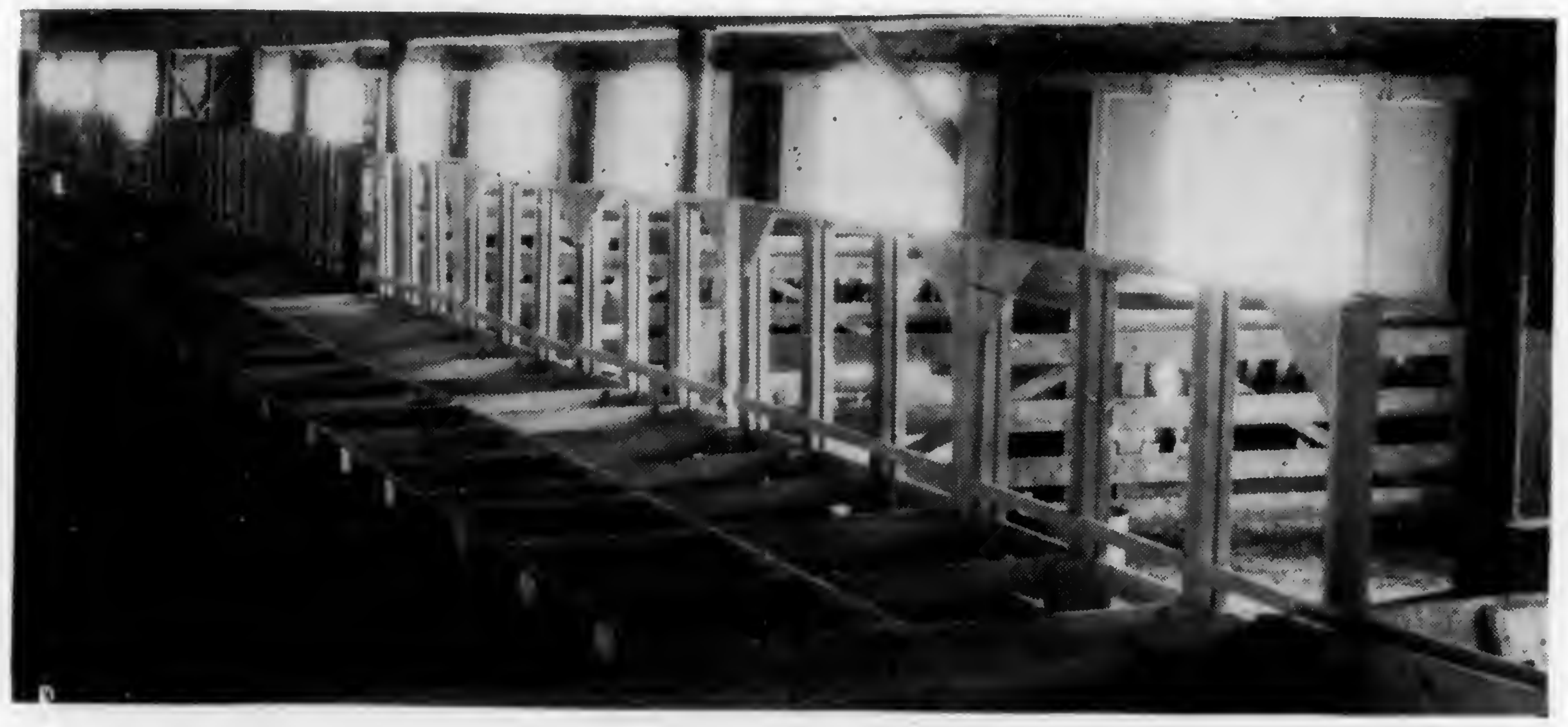


Fig. 1.—Experimental equipment. All lambs were fed in individual stalls which allowed for complete accounting of their feed.

If the lamb feeder desires to know the most economical ratio of concentrate to hay to feed with a ration of soybean oilmeal 35 parts, corn 64 parts, and hay, when the concentrate mixture costs $1.20 per hundred pounds and the hay costs $15 per ton, the point intersected on the intermediate scales will give the cost of 100 pounds of gain. With these prices, the cost of 100 pounds of gain according to Nomograph 1 was $6.10, $7.00, and $9.20 for the lambs fed rations with the concentrate to hay ratios of 3:2, 1:1, 2:3, respectively.

The cost of 100 pounds of gain of the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay as shown in Nomograph 2, was $6.75, $7.70, and $11.40 for the lambs fed the rations with the concentrate to hay ratios of 3:2, 1:1, and 2:3, respectively.

If the lamb feeder desires to know the most economical soybean oilmeal ration to feed regardless of the ratio of concentrate to hay, the cost of 100 pounds of gain of the lambs fed the ration of soybean oilmeal 35 parts, corn 64 parts, and hay, as given by Nomograph 1, may be compared with the cost of 100 pounds of gain of the lambs fed the ration of soybean oilmeal 10 parts, corn 89 parts, and hay, as given by Nomograph 2.

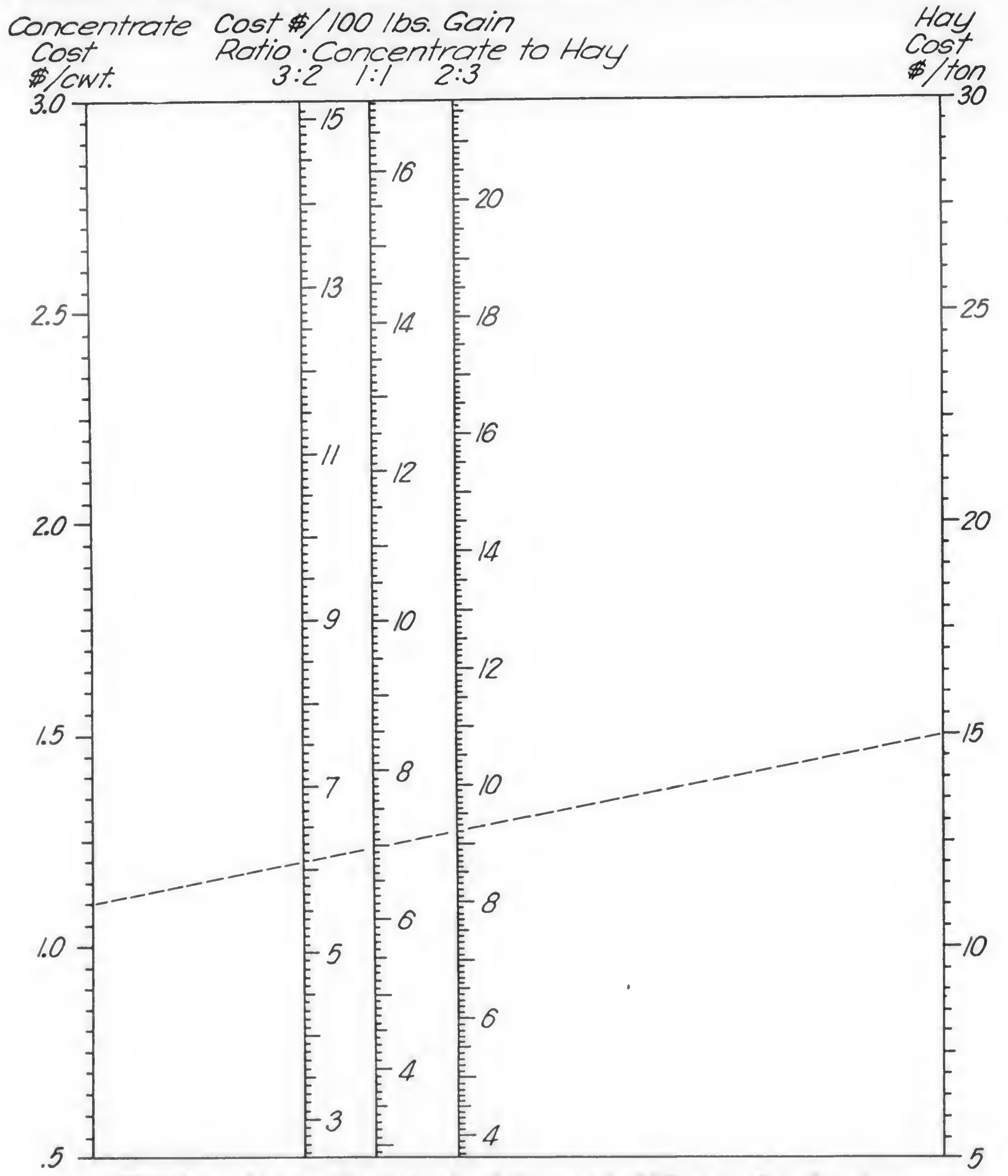
SUMMARY

Sixty-six pairs of lambs were fed in this study to determine the optimum percentage of soybean oilmeal that can be used as a source of protein in a concentrate mixture in combination with different proportions of hay for growing lambs. The following feeds were used: yellow corn, clover or mixed timothy-clover hay, and soybean oilmeal.

A study has been made of the effect of the ratio of concentrate to hay on the gains with varying quantities of soybean oilmeal. In three experiments the ratios of concentrate to hay among pairs were not controlled; the ratios varied between 1:1.1 and 1:1.8. In three experiments the ratios of concentrate to hay among pairs were kept constant throughout a group, the following ratios being studied: 3:2, 1:1, and 2:3.

The 5 per cent soybean oilmeal concentrate ration was found to be superior to corn alone when red clover hay of U. S. No. 3 grade

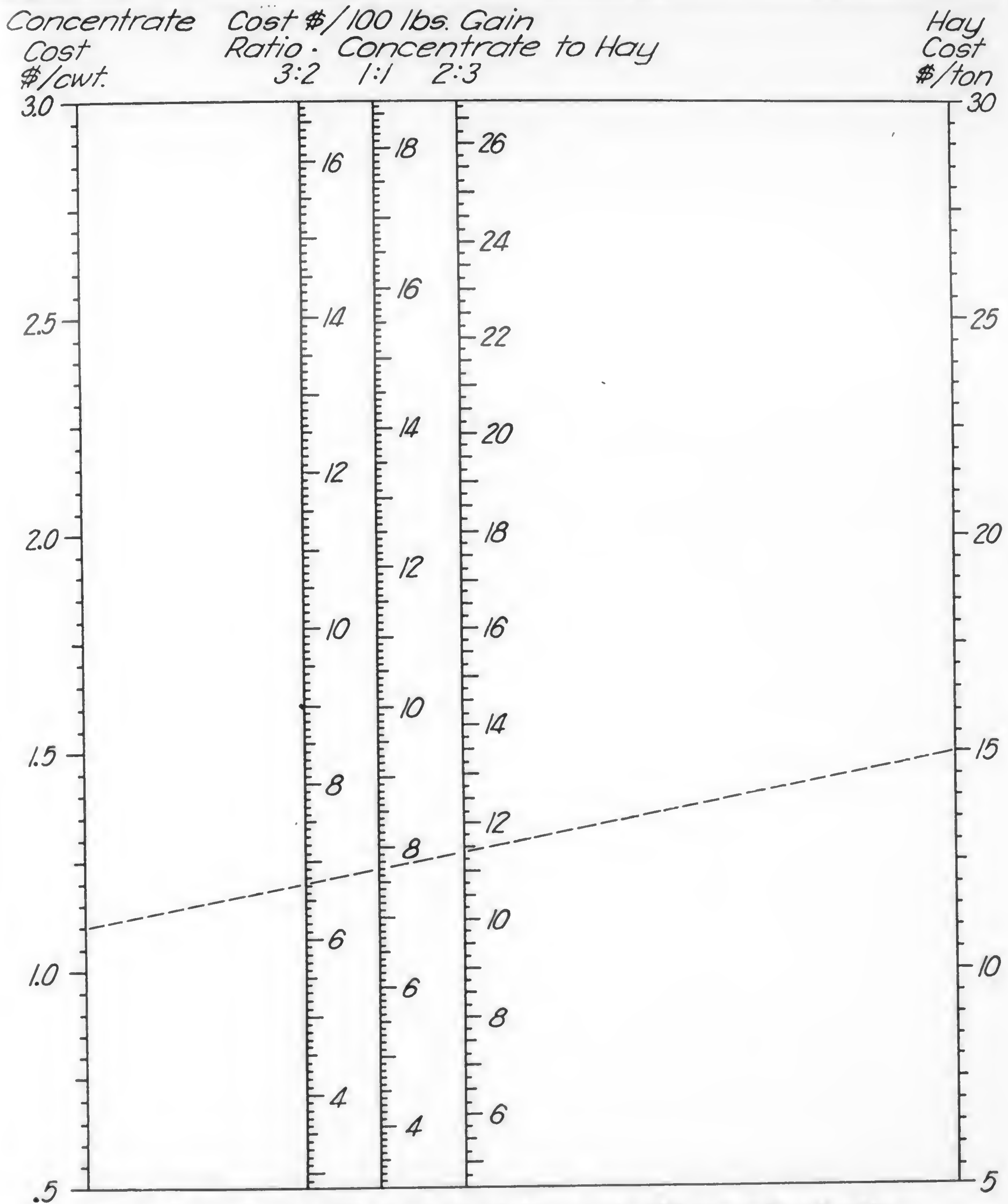
Nomograph 1: Used for the rapid calculation of feed cost of 100 pounds of gain of lambs fed hay and a concentrate mixture of soybean oilmeal 35 parts, corn 64 parts, for ratios of concentrate to hay 3:2, 1:1, and 2:3.



Feeds and quantity required for each 100 pounds of gain.

Feeds	Ratios of Concentrate to Hay		
	3:2 *pounds*	*1:1* *pounds*	*2:3* *pounds*
Corn	243.5	241.0	264.7
Soybean oilmeal	133.1	131.8	144.8
Salt (sodium chloride)	3.8	3.8	4.1
Hay	253.6	376.5	620.4
Total	634.0	753.0	1034.0
Per cent protein in ration	16.9	16.1	15.1

Nomograph 2: Used for the rapid calculation of feed cost of 100 pounds of gain of lambs fed hay and a concentrate mixture of soybean oilmeal 10 parts, corn 89 parts, for ratios of concentrate to hay of 3:2, 1:1, and 2:3.



Feeds and quantity required for each 100 pounds of gain.

Feeds	Ratios of Concentrate to Hay		
	3:2 *pounds*	*1:1* *pounds*	*2:3* *pounds*
Corn	373.8	369.4	455.7
Soybean oilmeal	42.0	41.5	51.2
Salt (sodium chloride)	4.2	4.1	5.1
Hay	280.0	415.0	768.0
Total	700.0	830.0	1280.0
Per cent protein in ration	12.6	11.9	11.9

was used. When U. S. No. 1 grade of timothy-clover mixture was used, a 5 per cent soybean oilmeal concentrate ration did not improve a corn ration, and a 10 per cent soybean oilmeal concentrate ration did not improve a 5 per cent soybean oilmeal concentrate ration.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and U. S. No. 1 red clover hay gained on the average 15 per cent more rapidly and required 13 per cent less feed than paired mates fed a ration of soybean oilmeal 10 parts, corn 89 parts, and red clover hay.

The lambs fed a ration with a concentrate-to-hay ratio of 2:3 required 46 and 73 per cent more feed per unit of gain than the lambs fed the rations with the concentrate-to-hay ratios of 1:1 and 3:2, respectively.

The lambs fed a ration with a concentrate-to-hay ratio of 3:2 required 19 and 74 per cent less feed per unit of gain than the lambs fed a ration with the concentrate-to-hay ratios of 1:1 and 2:3, respectively.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and red clover hay with the grain-to-hay ratio of 3: 2 made the most economical gains regardless of price of feeds. This ration contained 17 per cent protein.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and red clover hay made the most economical gains irrespective of the concentrate to hay ratio.

CONCLUSIONS

The lambs fed a ration of 3 parts of a concentrate mixture composed of 35 per cent soybean oilmeal, 64 per cent yellow corn and 1 per cent sodium chloride (common salt) and 2 parts of clover-timothy hay made the most economical and rapid gains from time of weaning until they reached 70 pounds liveweight. This ration contained 17 per cent protein.

The lambs fed a ration composed of 2 parts of a concentrate mixture of 10 per cent soybean oilmeal, 89 per cent yellow corn and 1 per cent sodium chloride and 3 parts of red clover hay made the most expensive gains. This ration contained only 12 per cent protein.

The low roughage rations were the most economical and produced the most rapid gains.

No detrimental effects were observed in the rations with high percentages of soybean oilmeal.

---

est of the consumer. In others, research is not much of a factor.

Some manufacturers regularly and painstakingly check every ingredient to make sure it meets specifications before it is used in the roofing. They maintain a careful system of production control while in others this is given very little importance and may result in material getting into the hands of the consumer before the irregularity is spotted.

Asphalt shingles are merely slate rolls cut into patterns.

Over a period of years, manufacturers have created a wide variety of designs and colors of shingles in order not to be outdone by a competitor. Unfortunately, sales effort has been focused on those shingle designs which make it easier for the contractor to bid low on a job or to show the consumer a price advantage. The line of least resistance has been chosen rather than the method of helping the consumer select a pattern which will give him proper weight, coverage, permanence of color, head lap and durability for the specific roof in question.

These were some of the findings which your organization made as it delved into this proposition.

With its own thoughts carefully correlated, the final check was a personal visit to the Bureau of Standards in Washington, D. C., where research, on an unbiased basis, has dealt with this subject for 13 years.

Selected types of roll roofing and shingles, designed to furnish value ir
ings, c
asph
b

Jacob Herr of L
Eastern St

The
Penı

Mr. Haley
phytochemistry
State College.

In re
fertilizer tre
vania cigar-
tions as to th

A p

obtain from manure
sources of supply. As
this element is quite
in the early stages o
considerable amount
should be in nitrate
form is immediately
the growing plant.
half to two-thirds
nitrogen should be
form, the remainder i
form such as cotto
from which nitrate n
be derived later in t
season.

It is known that
tolerate relatively l
ties of nitrogen u
conditions and with
preciable ill effects
standpoint of growth
standpoint of qualit
such a condition
avoided, as certain
trogen-containing co
the leaf are not broke
ing fermentation and
fere with the "burn"
and likewise the qu
smoke.

On the other hand
may and do arise w
suffer for want of
pecially in the earl
growth, notwithst

was used. When U. S. No. 1 grade of timothy-clover mixture was used, a 5 per cent soybean oilmeal concentrate ration did not improve a corn ration, and a 10 per cent soybean oilmeal concentrate ration did not improve a 5 per cent soybean oilmeal concentrate ration.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and U. S. No. 1 red clover hay gained on the average 15 per cent more rapidly and required 13 per cent less feed than paired mates fed a ration of soybean oilmeal 10 parts, corn 89 parts, and red clover hay.

The lambs fed a ration with a concentrate-to-hay ratio of 2:3 required 46 and 73 per cent more feed per unit of gain than the lambs fed the rations with the concentrate-to-hay ratios of 1:1 and 3:2, respectively.

The lambs fed a ration with a concentrate-to-hay ratio of 3:2 required 19 and 74 per cent less feed per unit of gain than the lambs fed a ration with the concentrate-to-hay ratios of 1:1 and 2:3, respectively.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and red clover hay with the grain-to-hay ratio of 3:2 made the most economical gains regardless of price of feeds. This ration contained 17 per cent protein.

The lambs fed a ration of soybean oilmeal 35 parts, corn 64 parts, and red clover hay made the most economical gains irrespective of the concentrate to hay ratio.

CONCLUSIONS

The lambs fed a ration of 3 parts of a concentrate mixture composed of 35 per cent soybean oilmeal, 64 per cent yellow corn and 1 per cent sodium chloride (common salt) and 2 parts of clover-timothy hay made the most economical and rapid gains from time of weaning until they reached 70 pounds liveweight. This ration contained 17 per cent protein.

The lambs fed a ration composed of 2 parts of a concentrate mixture of 10 per cent soybean oilmeal, 89 per cent yellow corn and 1 per cent sodium chloride and 3 parts of red clover hay made the most expensive gains. This ration contained only 12 per cent protein.

The low roughage rations were the most economical and produced the most rapid gains.

No detrimental effects were observed in the rations with high percentages of soybean oilmeal.

---

est of the consumer. In others, research is not much of a factor.

Some manufacturers regularly and painstakingly check every ingredient to make sure it meets specifications before it is used in the roofing. They maintain a careful system of production control while in others this is given very little importance and may result in material getting into the hands of the consumer before the irregularity is spotted.

Asphalt shingles are merely slate rolls cut into patterns.

Over a period of years, manufacturers have created a wide variety of designs and colors of shingles in order not to be outdone by a competitor. Unfortunately, sales effort has been focused on those shingle designs which make it easier for the contractor to bid low on a job or to show the consumer a price advantage. The line of least resistance has been chosen rather than the method of helping the consumer select a pattern which will give him proper weight, coverage, permanence of color, head lap and durability for the specific roof in question.

These were some of the findings which your organization made as it delved into this proposition.

With its own thoughts carefully correlated, the final check was a personal visit to the Bureau of Standards in Washington, D. C., where research, on an unbiased basis, has dealt with this subject for 13 years.

Selected types of roll roofing and shingles, designed to furnish value in usage on specific buildings, constitute the Eastern States asphalt roofing service which will be introduced soon through Exchange warehouses.

Further information on this subject will appear in future issues of the COOPERATOR. In the meantime, your fieldman, warehouse personnel or the Springfield office is prepared to discuss this subject with you.

Jacob Herr of Lancaster, Pennsylvania, fertilized his 1939 Pennsylvania cigar-leaf tobacco with *Eastern States 5-10-20* at the rate of 500 pounds to the acre. His results were excellent

The Fertilizer Needs of Pennsylvania Tobacco

By D. E. Haley

Mr. Haley is professor of soil and phytochemistry at the Pennsylvania State College.

IN recommending a given fertilizer treatment for Pennsylvania cigar-leaf tobacco, questions as to the quantity to be applied, the formula of the mixture and the form in which the fertilizing constituents occur, receive due consideration. Of possibly greater importance, however, is whether these constituents are available to the growing crop and at the proper time.

The tobacco crop makes a relatively heavy demand on many mineral nutrients during its short period of growth. This makes it imperative for every plant to possess a good root system, a point which was stressed in the November issue of the COOPERATOR.

It is fairly well recognized that the tobacco plant should be kept in a condition of active growth from the time the seed has germinated until the plant reaches maturity, insofar as it is humanly possible to do so. Aside from the physical condition of the soil and its available water supply, certain fertilizer constituents play an important role in this connection. A few of these may now be mentioned.

Nitrogen: Pennsylvania cigar-leaf tobacco appears to require from 30 to 40 pounds of nitrogen per acre in the form of commercial fertilizers, plus what it may

A partial view of the three-year rotation experiment at Roseville, Pennsylvania, in the spring of 1939

obtain from manure and other sources of supply. As the need for this element is quite pronounced in the early stages of growth, a considerable amount of nitrogen should be in nitrate form, as this form is immediately available for the growing plant. From one-half to two-thirds of the total nitrogen should be in available form, the remainder in an organic form such as cottonseed meal, from which nitrate nitrogen may be derived later in the growing season.

It is known that plants may tolerate relatively large quantities of nitrogen under certain conditions and without any appreciable ill effects from the standpoint of growth. From the standpoint of quality, however, such a condition should be avoided, as certain of the nitrogen-containing constituents of the leaf are not broken down during fermentation and later interfere with the "burn" of the leaf and likewise the quality of the smoke.

On the other hand, conditions may and do arise where plants suffer for want of nitrogen, especially in the early stages of growth, notwithstanding the fact that considerable quantities of nitrogen may have been added. The plowing under of old clover and alfalfa stubble, or the addition of fresh cow manure may induce such a condition, as the microorganisms involved in the decay of such materials may compete with the tobacco for the available nitrogen.

The continued utilization of nitrogen may be seriously interfered with, if not rendered impossible, in the case of a drought. In the case of an incipient drought, starch and other carbohydrates may be found in the leaf. In the case of a prolonged drought, this may not be true. Plants are only able to utilize nitrogen when starch and sugars are present. In certain forms, it may even prove deleterious when these compounds are lacking.

While a drought cannot be prevented, its harmful effects may be considerably reduced by following corn rather than clover or alfalfa with tobacco and using fairly well rotted rather than fresh manure wherever possible in addition to ample quantities of a well-balanced fertilizer mixture.

Potassium: For many years a number of our growers failed to use sufficient quantities of potash fertilizers, although in many areas this element appears to be a limiting factor of growth. The general improvement in the quality of the Pennsylvania tobacco crop, so noticeable in recent years, may be attributed in large measure to the extended use of potash fertilizers.

Although potassium is found as potash salts in the fertilizer mixture, which are soluble in water, very little of the potassium is found in a water-soluble form following an application of this element to the soil. If brought into contact with the roots of the plant, however, it may be absorbed. In large measure, the quantity of potassium absorbed is closely correlated to the extent of root development. This involves, among other things, good drainage, frequent cultivation, available moisture and a comparative freedom from organic residues which are slow to decompose, such as clover stubble.

The reasons why potassium is of paramount importance to the tobacco crop are not difficult to find. For example, it favors the formation and stability of proteins which are absolutely necessary to the health of the plants. In the absence of the necessary quantity, non-protein compounds may accumulate — a condition to be avoided.

In combination with citric and other acids, potassium contributes to the fermentability of the leaf, to its "burn" and to the sweetness of the smoke.

Potash fertilization and disease resistance, in large measure, appear to go hand-in-hand. On many occasions during the season of 1939, however, such a relationship did not appear to exist. These instances, however, may be definitely related to the lack of potash absorption. Notwithstanding the fact that large quantities may have been added to the soil, the plants involved showed potash deficiencies in every case observed, there not being enough soil moisture to facilitate absorption.

Our tobacco soils appear to require from 120 to 160 pounds of potash per acre. For best results, the potash content of the leaf should be equal or slightly in excess of the nitrogen content. A ratio of two parts of nitrogen to one of potassium is unsatisfactory from the standpoint of both yield and quality.

Phosphorus: Our soils appear to require approximately 80 pounds of phosphoric acid per acre in the form of commercial fertilizers. This quantity may at first be considered excessive as the crop itself does not contain a large quantity of this element. The beneficial effects of phosphoric acid, however, appear to be indirect as well as direct.

Fertilizer Recommendations: It has been recommended for the average farm in Lancaster county, the center of the cigar-leaf industry of Pennsylvania, that either a 3–8–12, a 4–8–12 or a 3–8–16 mixture may be used to advantage in quantities of 1000 pounds per acre to tobacco following corn in the rotation, with at least 10 tons per acre of farm manure in a fairly well-rotted condition wherever possible.

The ill effects of clover residues or fresh manure may be partially or wholly overshadowed where the temperature is optimum for growth and where the rainfall has been both ample and well distributed.

What Happens If Hybrid Sweet Corn Is Planted

By O. H. Pearson

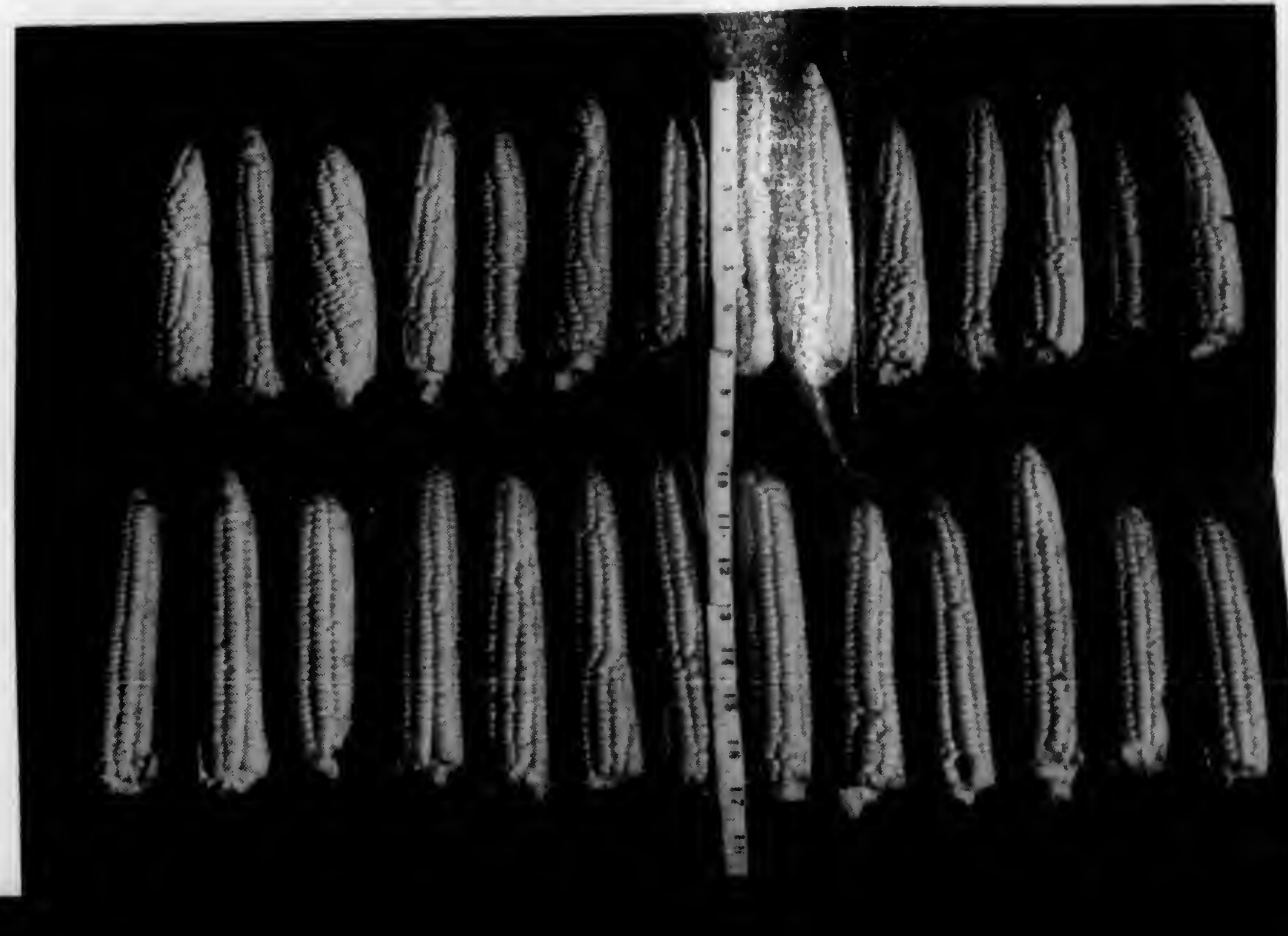
Dr. Pearson directs the Exchange's vegetable plant breeding operations, which include field trials in several sections.

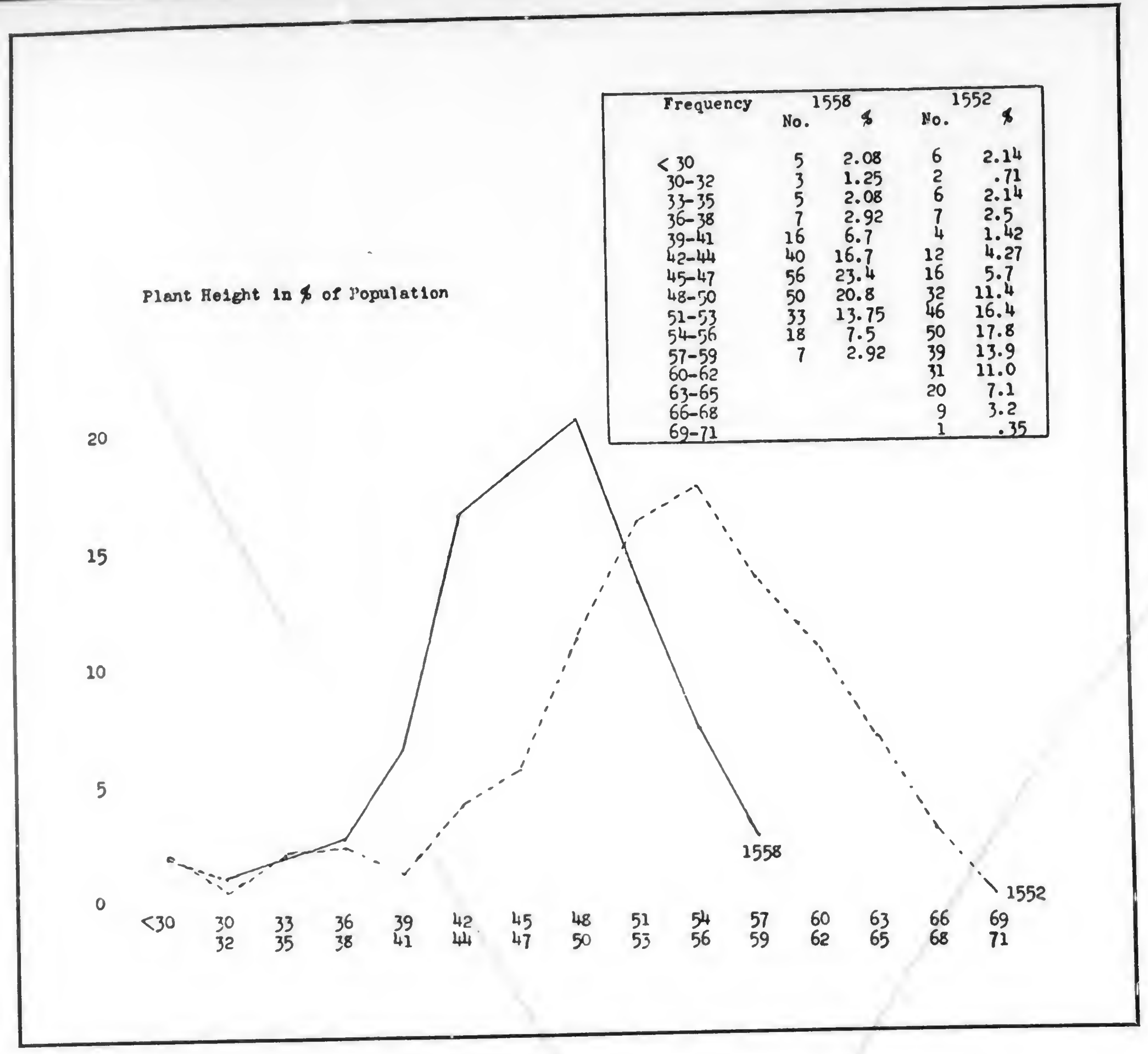
Hybrid sweet corn is becoming widely known for its uniformity, productivity and quality and the better hybrids will surpass standard varieties in all three counts. The word "hybrid" is literally the result of breeding together two unlike parents, and need not be applied only to the first cross. Later generations from a cross of two unlike parents, no matter how uniform is the immediate result (F1) of the cross, always "break up" or vary in the characters in which the parents differed. If enough individuals of such a second generation (F2) are observed, some that closely resemble each parent will be found, as well as a large number that are similar to the first generation (F1). Intergrades will make up the largest portion of the group and new forms not found in either parent or in the first generation hybrid may appear.

First generation hybrid sweet corn seed will produce a uniform crop of good quality ears, but as you can see, crops grown from seed saved even from the most uniform hybrid may be disappointing. Instances are known where seed has been saved from Golden Cross Bantam and the results were unsatisfactory, and similarly seed saved from other crosses produce crops that are inferior. There is a strong temptation to save seed from crosses which are exceptionally good and on which the supply of seed is limited, but it may be that the results will not justify planting such second generation seed.

In 1938 we heard of such a planting made by Arthur G. Low of Derry, New Hampshire, using seed saved from a field of *Marcross 6–13* the previous year. A visit to

These two rows of sweet corn ears show the results of Arthur G. Low's comparison of *Eastern States Marcross 6-13* (bottom row) and the product (top row) of seed saved from a crop of this hybrid. Uniformity being one of the virtues of quality sweet corn, note how this is dissipated by the "breaking up" of the hybrid in its second generation





Frequency	1558 No.	1558 %	1552 No.	1552 %
< 30	5	2.08	6	2.14
30-32	3	1.25	2	.71
33-35	5	2.08	6	2.14
36-38	7	2.92	7	2.5
39-41	16	6.7	4	1.42
42-44	40	16.7	12	4.27
45-47	56	23.4	16	5.7
48-50	50	20.8	32	11.4
51-53	33	13.75	46	16.4
54-56	18	7.5	50	17.8
57-59	7	2.92	39	13.9
60-62			31	11.0
63-65			20	7.1
66-68			9	3.2
69-71			1	.35

This very scientific looking graph shows the plant height characteristics of *Eastern States Marcross 6-13* in contrast to corn grown from a planting of seed saved from a crop of the same hybrid. Number 1552 is the hybrid's descendant, whereas 1558 is the hybrid, itself

this planting showed the danger which was involved in such a practice because the plants were uneven, the ears variable in length and the whole crop about on a par with many locally grown varieties. There were also a large number of barren plants present. Only a little of first generation seed was planted in this field, so no accurate comparison could be made.

Seed was collected in 1938 from a field of first generation *Marcross 6-13* and 100 feet each of this and of first generation *Marcross 6-13* were planted in adjacent rows at Feeding Hills, Massachusetts, in 1939. Moisture supply was ample, in spite of the dry season. Because of lack of space, one seed was planted every four inches. This was not expected to influence any of the characters to be measured, except possibly ear length.

There proved to be some difference in stand, as the first generation seed produced only 80 percent of expected plants, and the local seed produced 93 percent. This probably is due to the use of the inbred Conn. 6 as a seed parent in the case of the first generation, seed of which is distinctly smaller and possibly weaker. The kernels of the ear produced by this seed (and which were saved as second generation) are large, well filled, and possibly should show a better stand.

In the figure showing the height of each plant to the base of the tassel, there appear to be approximately two percent of each group less than 30 inches tall, and practically the same proportion in each from 30-38 inches tall. These smaller plants are quite possibly the result of attack by soil-borne diseases of the root system, or the normal occurrence of plants with defective root systems. The classification of the plants that were more than 39 inches tall show that the second generation plants were definitely taller than the first generation, probably approaching the type of one of the parent varieties, Whipple. Many of the plants were distinctly pale green. The two lots appeared to be quite unlike each other.

At maturity in the fall, all the ears were picked, measured, rows counted, and the number showing the dropping out of a pair of rows noted. Because the plants stood so close in the row, no weight can be given to results that might indicate yielding ability. Further study will have to be given this point. Certain differences did stand out. There was a difference in average ear length, the second generation averaging .85 inch shorter. Also, more than 75 percent of the ears of the first generation group are over 5.5 inches long, but less than 45 percent of the ears of the other group reached this length.

In the case of row number, over 70 percent of the ears of the first generation group fell in the 12-row class, and over 50 percent of the second group were in this class. There were a few more ears with larger row numbers in the second generation group. Also, there were twice as many ears with dropped out rows as in the first generation group. From these figures it would appear that the ears of the second generation group were more bulky and this is true as far as the ear itself is concerned. However, we observed that the husk covering on these ears is thinner than on first generation ears, and this together with their shorter length,

---

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1940, **44**, 203-207

11401

Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates.*

CLAIRE A. FREDERICK, N. B. GUERRANT, R. ADAMS DUTCHER AND C. A. KNIGHT.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

It is now recognized that the rat is not only able to subsist indefinitely on a diet that is markedly scorbutogenic to the guinea pig, but while doing so, stores in its tissues and excretes in its urine measurable amounts of ascorbic acid. Workers in this field are not in complete agreement regarding the relationship of the composition of the ingested diet to the amounts of ascorbic acid stored and excreted by the rat. Some investigators[1,2] have contended that the composition of the diet is an influencing factor, while other investigators[3-6] have failed to demonstrate that ascorbic acid output is affected by changes in dietary ingredients. Other investigators[7-10]

---

* Authorized for publication on March 16, 1940, as paper No. 962 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[1] Hopkins, F. G., Slater, B. R., and Milliken, G. A., *Biochem. J.*, 1935, **29**, 2803.

[2] Menaker, M. H., January, 1938, Master's Thesis, Pennsylvania State College.

[3] Svirbely, J. L., *Am. J. Physiol.*, 1936, **116**, 446.

[4] Zilva, S. S., *Biochem. J.*, 1936, **30**, 857.

[5] Scheunert, A., and Schieblich, M., *Z. Physiol. Chem.*, 1937, **247**, 272.

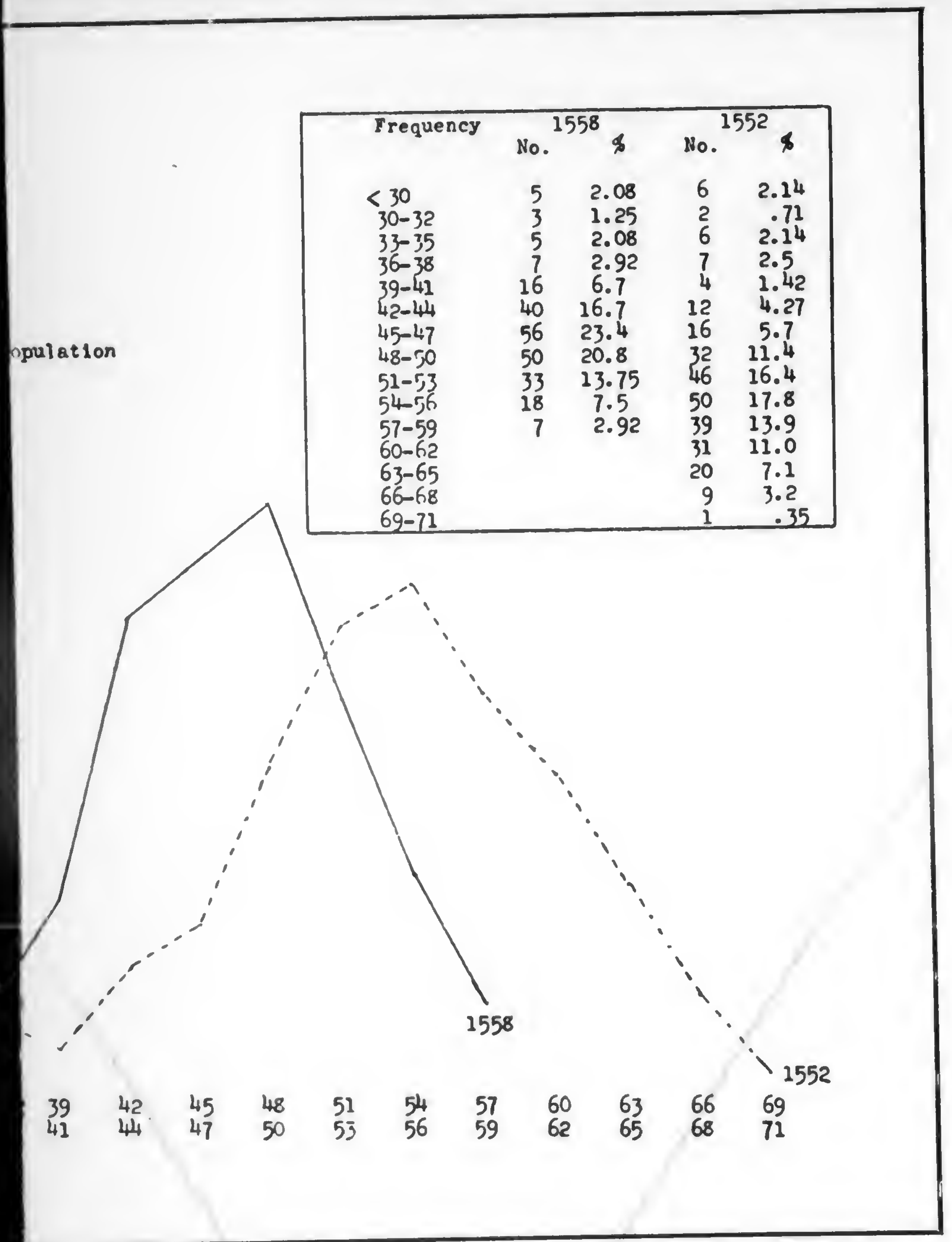
[6] Mentzer, C., and Urbain, G., *Compt. Rend. Soc. Biol.*, 1938, **128**, 270.

[7] Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *Science*, 1938, **88**, 552.

[8] Longenecker, H. E., Musulin, R. R., and King, C. G., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, 1939, **128**, p. lx.

[9] Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *J. Biol. Chem.*, 1939, **129**, 437.

[10] Longenecker, H. E., Musulin, R. R., Tully, R. H., 3rd, and King, C. G., *J. Biol. Chem.*, 1939, **129**, 445.



Frequency	1558 No.	1558 %	1552 No.	1552 %
< 30	5	2.08	6	2.14
30-32	3	1.25	2	.71
33-35	5	2.08	6	2.14
36-38	7	2.92	7	2.5
39-41	16	6.7	4	1.42
42-44	40	16.7	12	4.27
45-47	56	23.4	16	5.7
48-50	50	20.8	32	11.4
51-53	33	13.75	46	16.4
54-56	18	7.5	50	17.8
57-59	7	2.92	39	13.9
60-62			31	11.0
63-65			20	7.1
66-68			9	3.2
69-71			1	.35

This very scientific looking graph shows the plant height characteristics of *Eastern States Marcross 6-13* in contrast to corn grown from a planting of seed saved from a crop of the same hybrid. Number 1552 is the hybrid's descendant, whereas 1558 is the hybrid, itself

the danger
in such a
lants were
riable in
rop about
lly grown
lso a large
its present.
generation
is field, so
n could be

1938 from
n *Marcross*
of this and
rcross 6-13
nt rows at
husetts, in
vas ample,
n. Because
seed was
ches. This
luence any
measured,
th.
me differ-
st genera-
ly 80 per-
, and the
percent.

and possibly weaker. The kernels of the ear produced by this seed (and which were saved as second generation) are large, well filled, and possibly should show a better stand.

In the figure showing the height of each plant to the base of the tassel, there appear to be approximately two percent of each group less than 30 inches tall, and practically the same proportion in each from 30–38 inches tall. These smaller plants are quite possibly the result of attack by soil-borne diseases of the root system, or the normal occurrence of plants with defective root systems. The classification of the plants that were more than 39 inches tall show that the second generation plants were definitely taller than the first generation, probably approaching the type of one of the parent varieties, Whipple. Many of the plants were distinctly pale green. The two lots appeared to be quite unlike each other.

At maturity in the fall, all the

so close in the row, no weight can be given to results that might indicate yielding ability. Further study will have to be given this point. Certain differences did stand out. There was a difference in average ear length, the second generation averaging .85 inch shorter. Also, more than 75 percent of the ears of the first generation group are over 5.5 inches long, but less than 45 percent of the ears of the other group reached this length.

In the case of row number, over 70 percent of the ears of the first generation group fell in the 12-row class, and over 50 percent of the second group were in this class. There were a few more ears with larger row numbers in the second generation group. Also, there were twice as many ears with dropped out rows as in the first generation group. From these figures it would appear that the ears of the second generation group were more bulky and this is true as far as the ear itself is concerned. However, we observed

---

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1940, **44**, 203-207

11401

Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates.*

CLAIRE A. FREDERICK, N. B. GUERRANT, R. ADAMS DUTCHER AND C. A. KNIGHT.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

It is now recognized that the rat is not only able to subsist indefinitely on a diet that is markedly scorbutogenic to the guinea pig, but while doing so, stores in its tissues and excretes in its urine measurable amounts of ascorbic acid. Workers in this field are not in complete agreement regarding the relationship of the composition of the ingested diet to the amounts of ascorbic acid stored and excreted by the rat. Some investigators[1,2] have contended that the composition of the diet is an influencing factor, while other investigators[3-6] have failed to demonstrate that ascorbic acid output is affected by changes in dietary ingredients. Other investigators[7-10] have contended that the ordinary constituents of the diet, such as sugar, fats and proteins, have no effect on the urinary excretion of ascorbic acid by the rat, but that high rates of excretion can be induced by feeding oats, oat oil, the unsaponifiable portion of oat oil, halibut liver oil and certain cyclic compounds of the terpene and sesqui-terpene series. Early reports by this group of investigators postulated the existence of a precursor from which the ascorbic acid was formed by the rat. In the later reports, however, these authors abandon this view and postulate that the ascorbic acid is formed through intermediary metabolism, a view previously expressed by other investigators.[6]

---

* Authorized for publication on March 16, 1940, as paper No. 962 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[1] Hopkins, F. G., Slater, B. R., and Milliken, G. A., *Biochem. J.*, 1935, **29**, 2803.

[2] Menaker, M. H., January, 1938, Master's Thesis, Pennsylvania State College.

[3] Svirbely, J. L., *Am. J. Physiol.*, 1936, **116**, 446.

[4] Zilva, S. S., *Biochem. J.*, 1936, **30**, 857.

[5] Scheunert, A., and Schieblich, M., *Z. Physiol. Chem.*, 1937, **247**, 272.

[6] Mentzer, C., and Urbain, G., *Compt. Rend. Soc. Biol.*, 1938, **128**, 270.

[7] Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *Science*, 1938, **88**, 552.

[8] Longenecker, H. E., Musulin, R. R., and King, C. G., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, 1939, **128**, p. lx.

[9] Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *J. Biol. Chem.*, 1939, **129**, 437.

[10] Longenecker, H. E., Musulin, R. R., Tully, R. H., 3rd, and King, C. G., *J. Biol. Chem.*, 1939, **129**, 445.

Because of the conflicting reports concerning the possible origin of ascorbic acid in the body of the rat, as well as those concerning factors influencing its elimination, it seemed desirable that our previous studies be repeated, especially those relating to the carbohydrate portion of the diet. The present report contains some of the data obtained in the course of the latter investigation.

Experimental. In our studies we have used half-grown rats as the experimental subjects. In order to collect the urine quantitatively, the animals were maintained in individual, cylindrical, galvanized wire cages, each of which was suspended above a 10-inch glass funnel. Beneath the funnel was placed a small glass vessel containing, as a preservative, 4 ml of metaphosphoric acid solution (10%) to which had been added a trace of 8-hydroxy-quinolin and a one-fourth-inch layer of paraffin oil. The cages were provided with galvanized wire bottoms with mesh of sufficient size to allow all fecal particles to pass through. Under each cage was placed a finer galvanized wire screen to prevent the fecal matter from entering the funnel. In order to further minimize contaminants, which might enter the funnel, the cages were provided with special food cups and drinking fountains. Each cage and its supplementary equipment were cleaned thoroughly at weekly intervals and, when necessary, the funnels were changed daily.

The collections of urinary samples were begun as soon as the animals were transferred to the metabolism cages and fed the experimental diet. The 24-hour collections of urine were removed at a definite time each day, their volumes recorded and aliquots titrated with Na 2,6-dichlorobenzenone indophenol solution (175 mg of the dye in 500 ml of hot water).

The diets used in these studies were composed of fat-free casein 18, salt mixture 3, Cell U flour 2, fat-free yeast 8 and fat-free carbohydrate 77 parts. In the majority of experiments, the fat-soluble

vitamins were furnished as beta carotene and calciferol. In a few instances these vitamins were supplied by adding 2 parts of cod liver oil to the basal diet. The carbohydrates used were: dextrinized corn starch, raw corn starch, sucrose, glucose, and in a limited number of feeding periods of short duration, mannose, sorbose, fructose and lactose. The amount of food consumed daily by each animal was

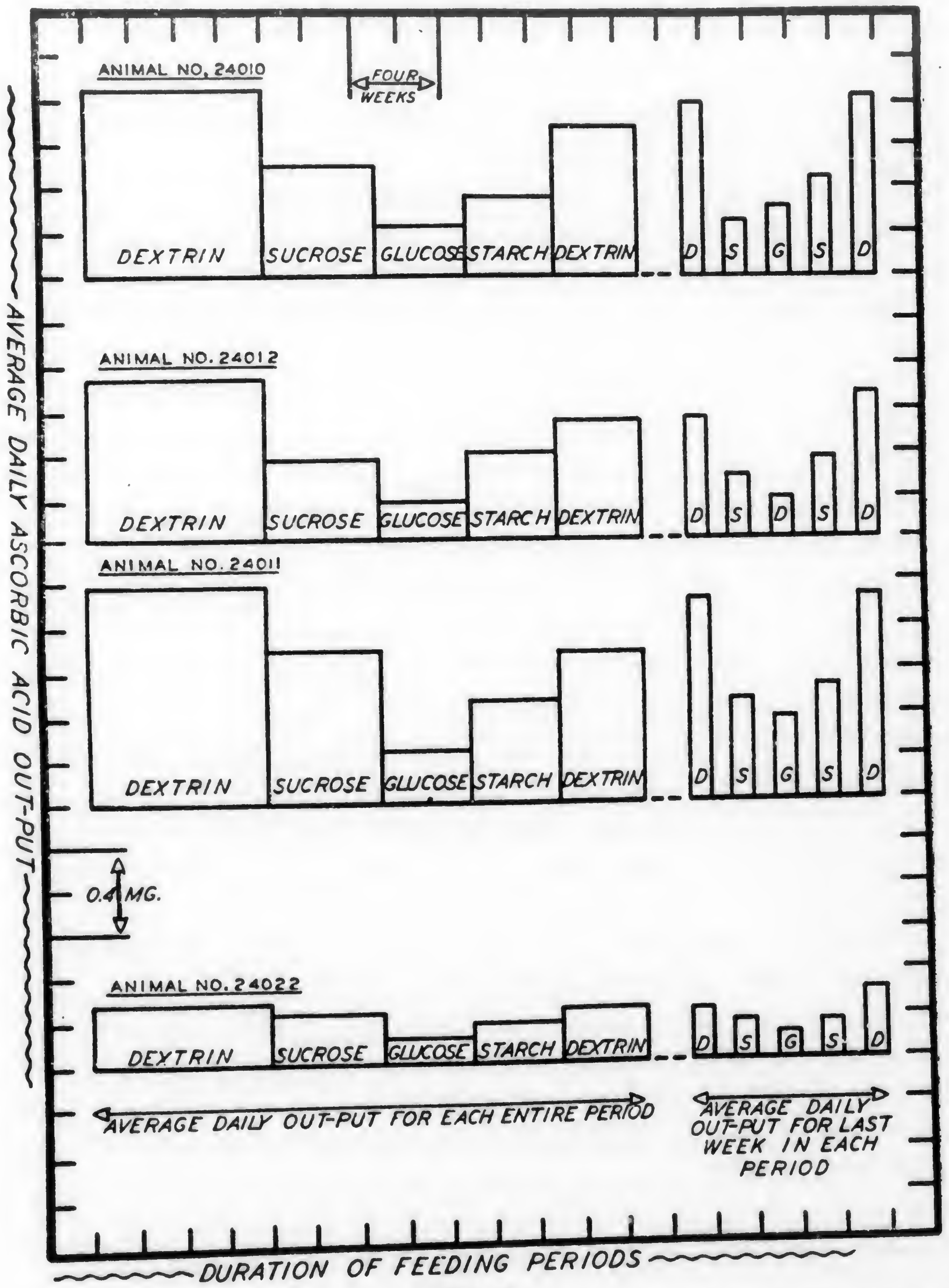


Fig. 1.

Amounts of ascorbic acid eliminated in the urine of rats while receiving diets similar in composition, the only difference being in the type of carbohydrate which they contained.

recorded and the amount fed was only slightly in excess of that consumed during the previous 24 hours.

In the instance of the first series of animals, all animals were fed the diet containing the dextrinized corn starch during the first period. This was followed by the sucrose-containing diet, the glucose-containing diet and the starch-containing diet, respectively. At the termination of the studies with these diets, the animals were again fed the dextrinized corn starch diet for a period of several weeks. With subsequent series of animals, diets of similar composition were used, the experimental difference being in the sequence in which they were fed to the test animals.

Since space does not permit the presentation of all the data at this time, only the condensed data relating to the urinary ascorbic acid output of 4 typical animals from the first series are given

The duration of the various feeding periods were 8, 5, 4, 4 and 4 weeks, respectively. The urinary ascorbic acid elimination has been expressed as the average daily output for the entire period during which each of the respective diets was fed. In order to indicate the probable carry-over effect of one diet on the succeeding diet, the average daily urinary ascorbic acid output for the last week of each feeding period is given on the right of the graph (Fig. 1).

Discussion. With all of the animals used, the urinary ascorbic acid output was greatest while the animals were consuming the diet containing the dextrinized corn starch. This was found to be true irrespective of the sequence in which the diets were fed. On the other hand, in most instances, these same animals eliminated the smallest quantity of ascorbic acid while consuming the glucose-containing diet. However, the frequency of the latter observation seemed to depend somewhat on the sequence in which the glucose diet was fed.

The greater ascorbic acid elimination resulting from the consumption of the diet containing the dextrinized corn starch as compared to the other diets, does not appear to be readily explainable from the data at hand. While the average daily consumption of the dextrinized corn starch was slightly greater than the consumption of the other diets and while there seemed to be a slight correlation between daily food intake and the amount of urinary ascorbic acid, this observation is sufficient only to explain a small portion of the increase in ascorbic acid elimination. The inadequacy of such an explanation can be readily observed from the fact that animal No. 24022 consumed as much of the respective diets as did animal No. 24011 and, while doing so, eliminated only about one-third as much urinary ascorbic acid as did the latter animal. However, it may be stated that the relative amounts of ascorbic acid eliminated by the various animals while receiving the sequence of diets were of the same order of magnitude for the respective diets.

The question as to why greater amounts of ascorbic acid were excreted, while the rats were consuming the dextrinized corn starch diet, than when the raw corn starch diet was consumed, cannot be answered at this time. The possibility of the existence of different amounts of an ascorbic acid precursor in the two diets does not seem tenable, at least in this instance. To verify this point, some of the rats, after having been returned to the dextrinized corn starch diet for several weeks, were given weighed amounts (25, 50 or 100 mg) of carvone (Eastman No. 1094) to determine the effect of this substance on ascorbic acid output. Since it was found impossible to determine, quantitatively, the amount of this volatile substance actually consumed by the rats when it was mixed with the diet, the carvone was diluted with olive oil and given by stomach tube. However, with this procedure, the tests proved unsatisfactory in that the animals began to lose weight after the administration of the first or the second dose of carvone and the majority of the test animals died within 6 or 7 days. In no case was there more than a twofold increase in the amount of ascorbic acid eliminated in any one day and such increases did not remain consistent from day to day.

Conclusions. It is apparent that the amount of ascorbic acid eliminated in the urine of the rat depends upon at least 2 major factors, namely, the type of carbohydrate ingested by the rat, and the physiological variations within the rat itself. Since it was frequently found that ascorbic acid elimination varied as much with different animals as it did with different diets, it is evident that the latter factor must be given due consideration. The data submitted, however, do not explain the origin of the ascorbic acid nor do they explain the differences in the amounts of ascorbic acid excreted in the urine of different animals while receiving comparable amounts of the same diet. A number of theoretical possibilities suggest themselves but these offer no immediate solution to the problem. It would serve no useful purpose to say that the ascorbic acid is probably of endogenous origin or that it has its origin in intermediate metabolites. Such suggestions fall far short of explaining the origin of the ascorbic acid in the body of the animal and would be equally ineffective in explaining why different animals excreted different amounts of this substance while consuming comparable portions of the same diet.

Reprinted from the PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1940, **44**, 90-93

11364

Destruction of Ascorbic Acid in the Rumen of the Dairy Cow.*

C. A. KNIGHT, R. A. DUTCHER, N. B. GUERRANT AND S. I. BECHDEL.

From the Department of Agricultural and Biological Chemistry, and the Department of Dairy Husbandry, The Pennsylvania State College, State College, Pa.

Dutcher and coworkers,[1] Hart, Steenbock and Ellis,[2] and Hess, Unger and Supplee[3] presented evidence to show that the diet of a dairy cow influenced the antiscorbutic potency of the milk produced. Using guinea pigs to test the antiscorbutic potency of the ration which was fed and to assay the milk which was produced, each of these groups of workers found that milk obtained from cows on a vitamin-rich ration was definitely superior in antiscorbutic value to the milk derived from cows on a vitamin-poor diet. These findings, though widely accepted, were disputed by Hughes and coworkers,[4] who concluded from a series of experiments that the ration received by cows had no influence on the antiscorbutic property of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, differences of opinion have arisen concerning the factors which have the greatest influence on the amount of vitamin C in milk. It is now generally agreed, however, that the vitamin C content of milk is independent of the season of the year and the ration of the cow.[5] This fact has led to the present investigation of the fate of ingested ascorbic acid in the cow.

---

* Authorized for publication on March 19, 1940, as paper No. 963 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[1] Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mead, S. W., and Schaffer, O. G., *J. Biol. Chem.*, 1920, **45**, 119.

[2] Hart, E. B., Steenbock, H., and Ellis, N. R., *J. Biol. Chem.*, 1920, **42**, 383.

[3] Hess, A. F., Unger, L. J., and Supplee, G. C., *J. Biol. Chem.*, 1920, **45**, 229.

[4] Hughes, J. S., Fitch, J. B., and Cave, H. W., *J. Biol. Chem.*, 1921, **46**, L.

[5] Kon, S. K., *The Journal of Dairy Research*, 1938, **9**, 242.

A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed (A) 100 g (2,000,000 International Units) and (B) 150 g of synthetic ascorbic acid mixed with corn silage; 100 g of ascorbic acid were also placed directly in the rumen through the fistula opening.

Similar results were obtained in all of the experiments. No increase was observed in the ascorbic acid values of the blood plasma and of the milk when compared with those values obtained while the cow was on a standard ration unsupplemented with the vitamin. A slight increase was noticed in the amount of ascorbic acid found in the 24-hour sample of urine for the periods during which the vitamin was administered.

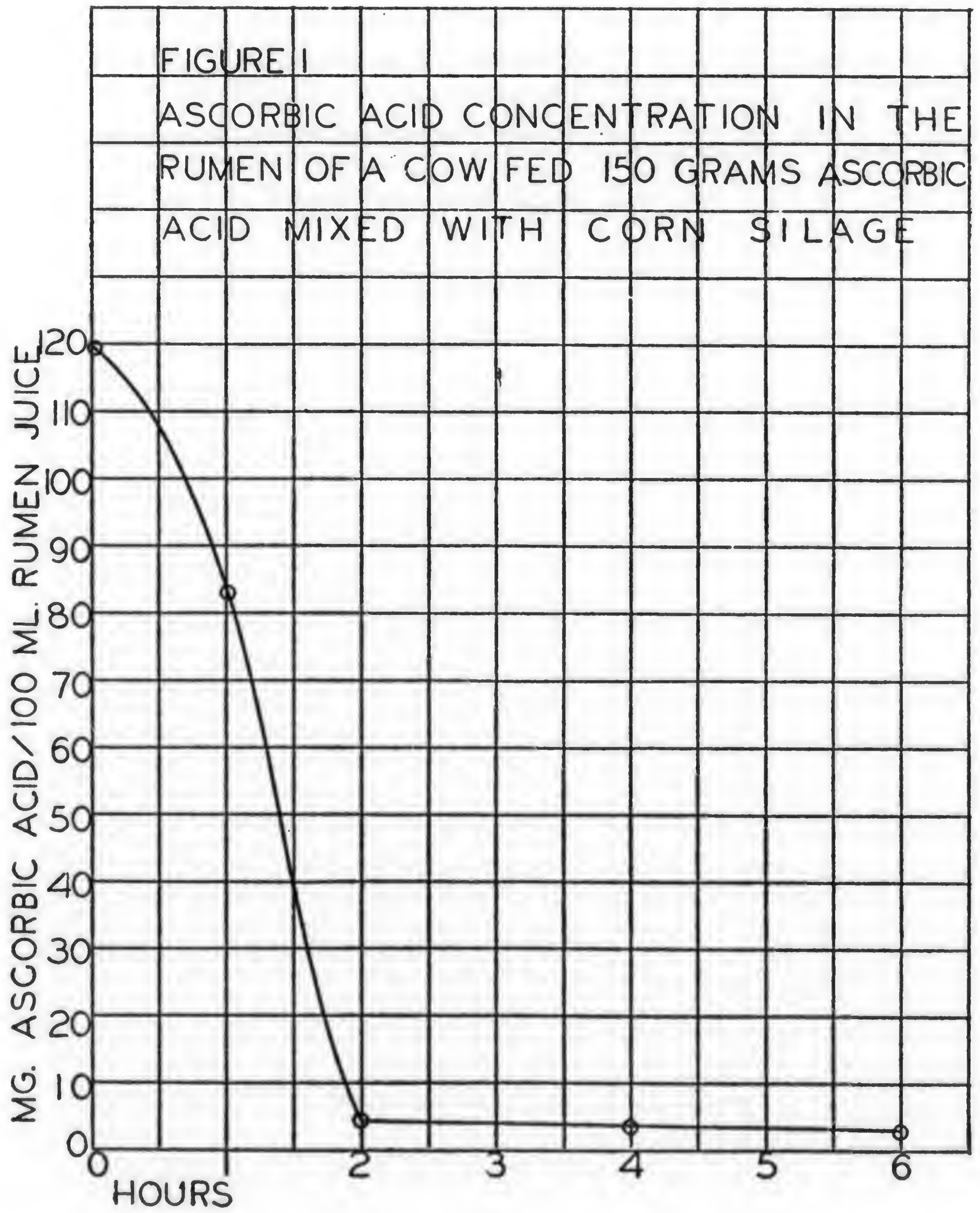


FIGURE I

ASCORBIC ACID CONCENTRATION IN THE RUMEN OF A COW FED 150 GRAMS ASCORBIC ACID MIXED WITH CORN SILAGE

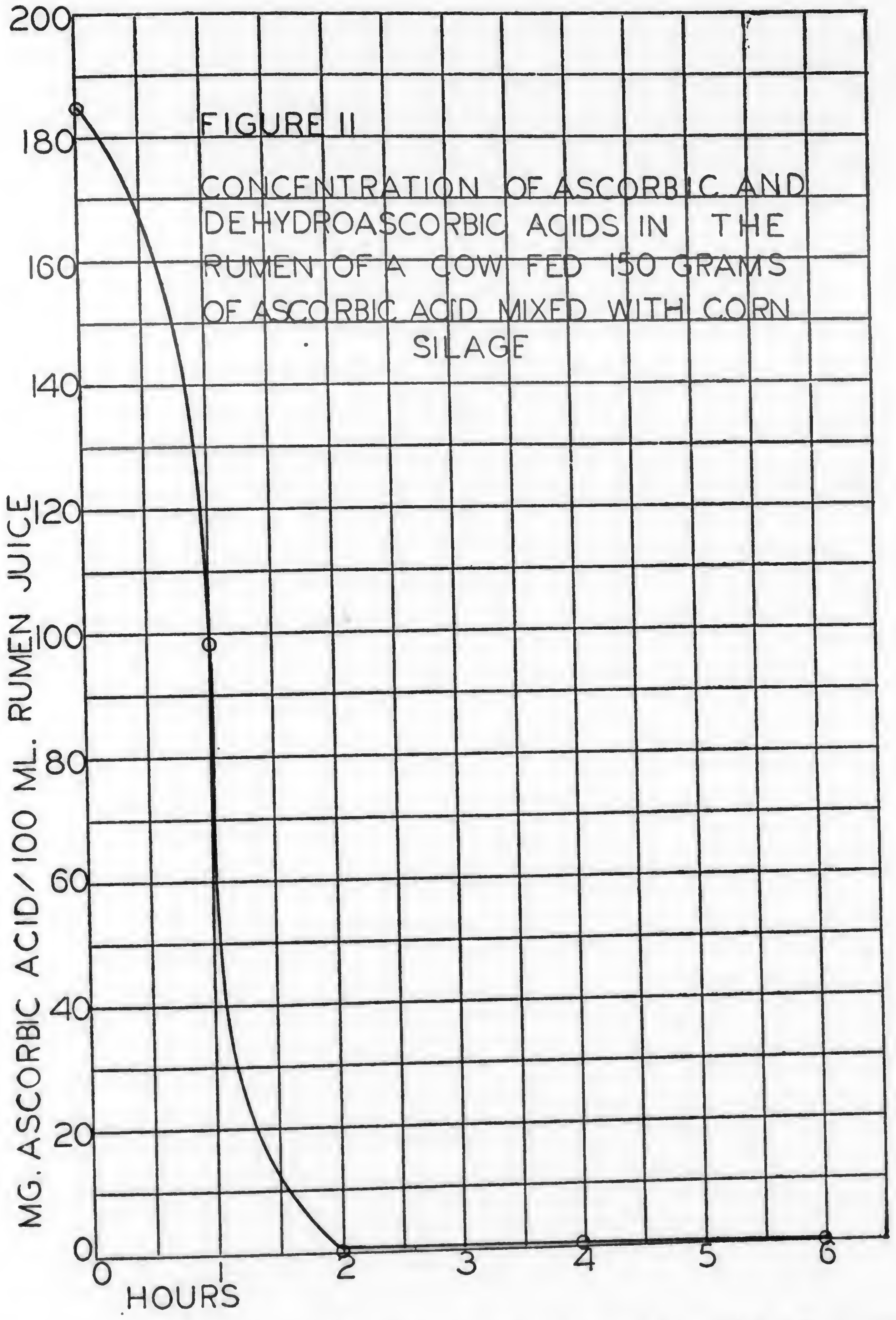


FIGURE II

CONCENTRATION OF ASCORBIC AND DEHYDROASCORBIC ACIDS IN THE RUMEN OF A COW FED 150 GRAMS OF ASCORBIC ACID MIXED WITH CORN SILAGE

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after the cow had been fed (Figs. 1 and 2). Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered bottle at 39°-42°C disappeared at much the same rate as that of the *in vivo* experiments.

These results are not in accord with the conclusions of Riddell and Whitnah[6] who suggested that the rapid disappearance of vitamin C from the rumen of a cow fed large amounts of green rye was due to a quick absorption of the vitamin.

In making the above analyses, both the indophenol titration and the Roe furfural method were employed.[7] The latter method was useful in detecting dehydroascorbic acid as well as the reduced form of the vitamin.

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

---

[6] Riddell, W. H., and Whitnah, C. H., *J. Dairy Science*, 1938, **21**, 121.

[7] Roe, J. H., and Hall, J. M., *J. Biol. Chem.*, 1939, **128**, 329.

Reprinted from The Journal of Biological Chemistry
Vol. 135, No. 2, September, 1940

THE EFFECTS OF HIGH PRESSURE ON THE ACTIVITY OF PEPSIN AND RENNIN

By JASON E. MATTHEWS, Jr., R. B. DOW, and ARTHUR K. ANDERSON

(From the Department of Agricultural and Biological Chemistry and the Department of Physics, the Pennsylvania State College, State College)

(Received for publication, June 4, 1940)

The general effects of high hydrostatic pressure on physical and chemical systems have been studied intensively by Bridgman (1) and by Cohen (2) over a pressure range of several thousand atmospheres. Considerable work has been done on the various effects of high pressure on biological systems. Bridgman (3) and Bridgman and Conant (4) studied denaturation by pressure of egg albumin and solutions of carboxyhemoglobin. Basset and his associates (5–9) reported the effect of high pressure on the activities of trypsin, diastase, sucrase, pancreatic lipase, and trypsinogen, as well as the use of pressure to control the toxicity of various sera, toxins, and venoms. Other investigations by Frankel and Meldolesi (10), Hite, Giddings, and Weakley (11), Larson, Hartzell, and Diehl (12), and Dow and Matthews (13) reported the behavior of enzymes, bacteria, and bovine blood, respectively, under similar conditions.

In this paper we are reporting on the effect of high pressure on the activity of pepsin and rennin. In the case of the former, crystalline pepsin was used. Since this is known to be a protein, the work with this enzyme should have double significance. Besides showing the effect of pressure on a typical enzyme, it should also throw some light on the denaturation of a pure protein as measured by an important biological property.

Methods

The method of Northrop (14) was used for the preparation of crystalline pepsin, and that of Anson and Mirsky (15) for the measure of its activity. The peptic unit of these authors has been used as the unit of activity for the results computed from the data of this paper. The rennin solution was prepared by dissolving 100 mg. of a dry enzyme preparation (No. 259, The Wilson Laboratories) in 100 cc. of distilled water with a small crystal of thymol for a preservative. The substrate was prepared from milk powder to insure consistent and reproducible coagulation times. 50 gm. of whole milk powder (Klim, The Borden Company) was dissolved in 500 cc. of distilled water and 5 cc. of an 8 per cent solution of $CaCl_2$ added. 200 cc. of boiling water were further added to the mixture and, after cooling, 1 volume of 0.02 M sodium acetate-acetic acid buffer solution (pH 6.2) was added to 3 volumes of the milk. The method used for determining activity was a slight modification of those of Tauber and Kleiner (16) and Stone and Alsberg (17). A standard test solution of rennin was considered as one that clotted a 10 cc. sample of the milk in 10 minutes at 40°. Both enzyme solutions were freshly prepared prior to each pressure treatment.

The method of generating high hydrostatic pressure is that of Bridgman (1). Pressures were measured in the test chamber by observing the change of electrical resistance with pressure of a coil of manganin wire, previous work having shown that the change of resistance varies linearly with change of pressure.

The pressure treatments of the pepsin solutions were applied at a constant temperature of 35.5° by means of a thermostatically controlled water bath that surrounded the test chamber, but the treatments for the rennin solutions were performed at room temperature (23°) without precautions to keep a constant temperature. The activity of the first is specified (15) at the above temperature but that of the second is not supposed to be critically dependent on temperature over a moderate range.

10 cc. samples of the solutions were prepared for the pressure treatment by being placed in paraffin-lined containers that separated the samples from the surrounding liquid which transmitted the pressure in the test chamber. One type of container, a flexible tooth paste tube, proved to be very effective in providing mechanical separation of the liquids, although it was found that aseptic conditions could be maintained and separation obtained equally well by placing the samples in cylindrical tubes and covering with a thick layer of sterilized paraffin oil. Basset (5) and Hite *et al.* (11) stated that no effects were found in their results that were due to the nature of the containers they used, and the writers have obtained reproducible data by using both types of the containers that have been described.

Pressure was always applied isothermally, at such a rate that 5 or 10 minutes were required to generate a few thousand atmospheres of pressure. Accordingly, the maximum change of temperature due to generation or release of pressure can be considered to be a fraction of a degree.

Results

The principal experimental results are shown by Figs. 1 to 5. Peptic and rennin activities have been measured as functions of pressure and exposure time, at constant concentrations and temperatures. It appears from the nature of the curves that a simple interpretation is impossible on the basis of these data alone. However, it seems desirable to call attention to some of the significant characteristics of the curves.

Bridgman and Conant (4) found the denaturation of carboxyhemoglobin by pressure to be a first order reaction dependent on the pH of the solution. It is evident from the data presented here (Figs. 1, 2, and 3), at constant pressure and temperature, that the reaction rate is not of the first order. Moreover, as regards the data for peptic activity we have found in come cases that the activity depended on the buffer and its pH. The pH of the standard solution was 4.8. When a potassium chloride-hydrochloric acid buffer of pH 2.2 was used, no change of activity from that of the standard unbuffered solution over a pressure range of 6000 kilos per sq.cm. was found. But with a potassium-hydrogen phthalate buffer of pH 4.2, a decrease of 45 per cent from that of the unbuffered solution was measured. Rennin activity over a range of 1000 kilos per sq.cm. appeared to change in a buffered sodium acetate-acetic acid solution (pH 6.2) by the same amount as in the unbuffered solution of pH 5.5.

Peptic activity was practically unaffected by pressures of 1000 kilos per sq.cm. or lower, over an exposure time interval of 6 hours. The activities of both enzyme solutions under pressure were found to be functions of the temperature. A detailed study of the tem-

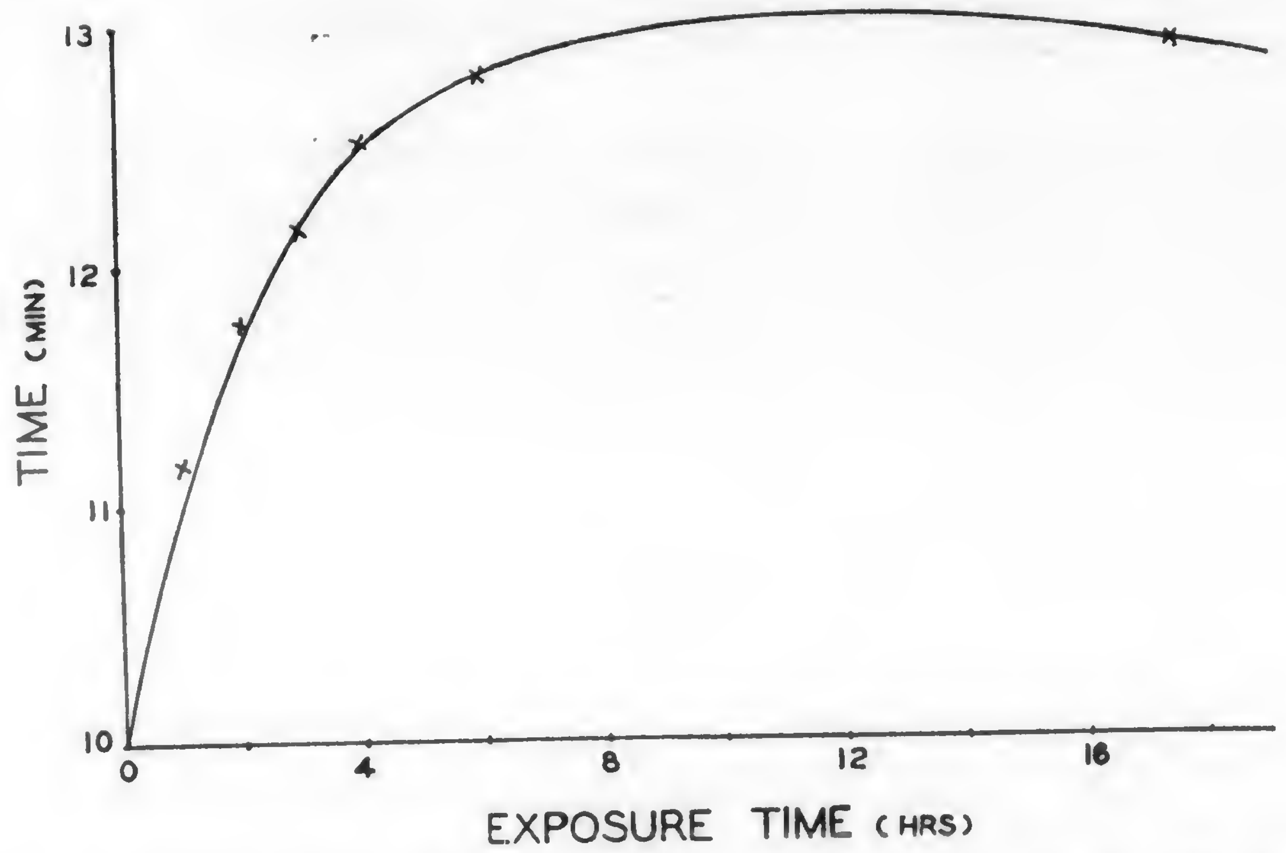


Fig. 1. Clotting time of rennin *versus* exposure time at 1000 kilos per sq.cm. The clotting time of the control was 10 minutes in every case.

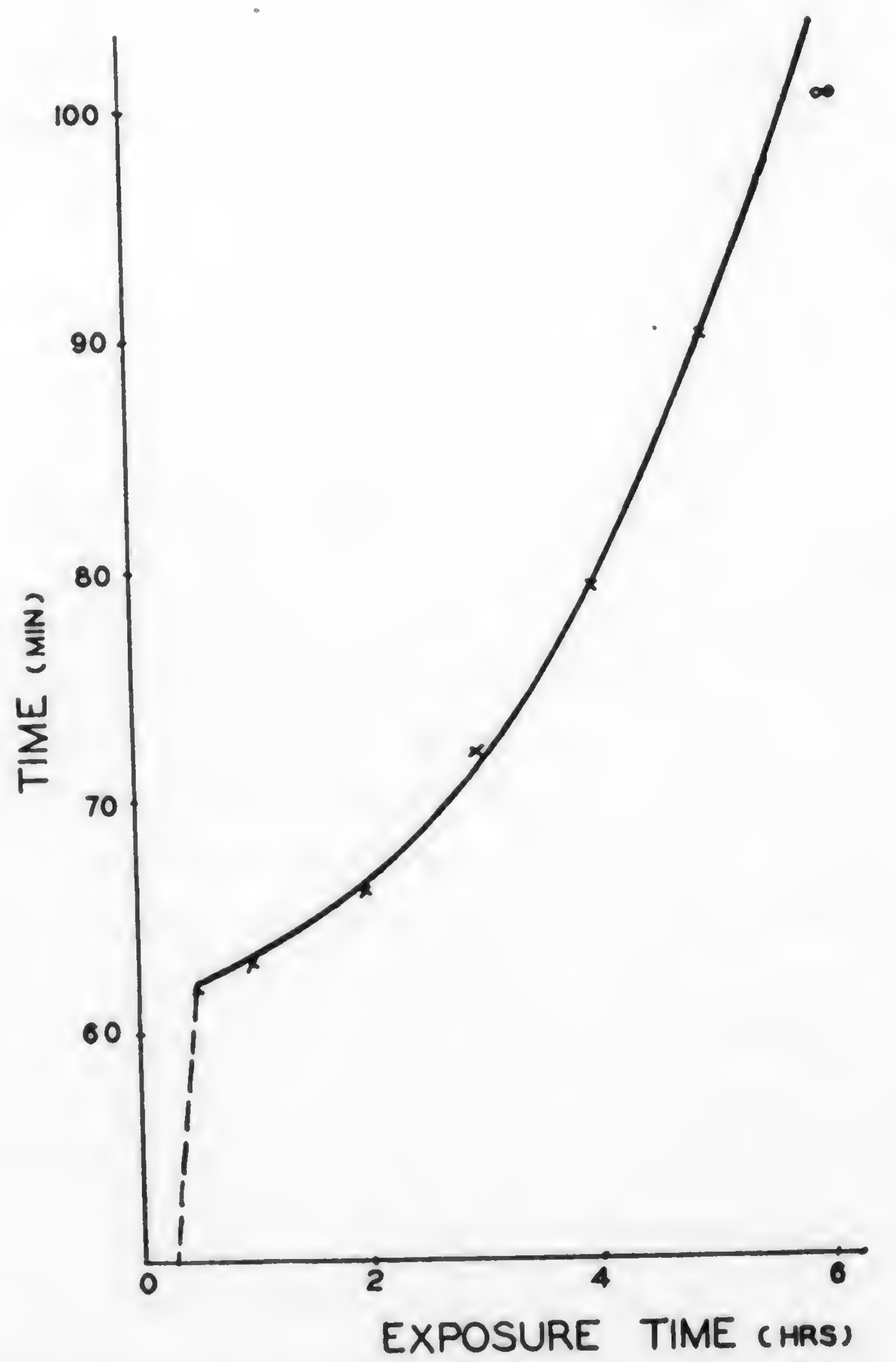


Fig. 2. Clotting time of rennin *versus* exposure time at 5000 kilos per sq.cm. The clotting time of the control was 10 minutes in every case.

perature effect was not made. For a pressure exposure of 5000 kilos per sq.cm. for 5 hours, the decrease of peptic activity was 25 per cent at 0° against 60 per cent at 35.5°. For rennin under similar conditions of pressure treatment, it was found that at 0° the coagulation time increased by 550 per cent, but at 23° the increase was 800 per cent, the activities varying inversely with the coagulation times.

The complicated variation of the activity *versus* exposure time curves at constant pressure suggests the existence of more than one reaction. In Fig. 3, for pepsin, particularly as regards the curve at 4500 kilos per sq.cm., it is to be noticed that the slopes of the curves changed in sign as the exposure time interval increased. Figs. 1 and 2 for rennin show strikingly the effect of pressure on the clotting time. It is evident that the activity of rennin as a function of time at 1000 kilos per sq.cm. is quite different from that at 5000 kilos per sq.cm. Accordingly, for both enzymes, it appears to us that several reactions probably with quite different rates occurred during the exposure interval, and that these rates are influenced strongly by the magnitude of the pressure.

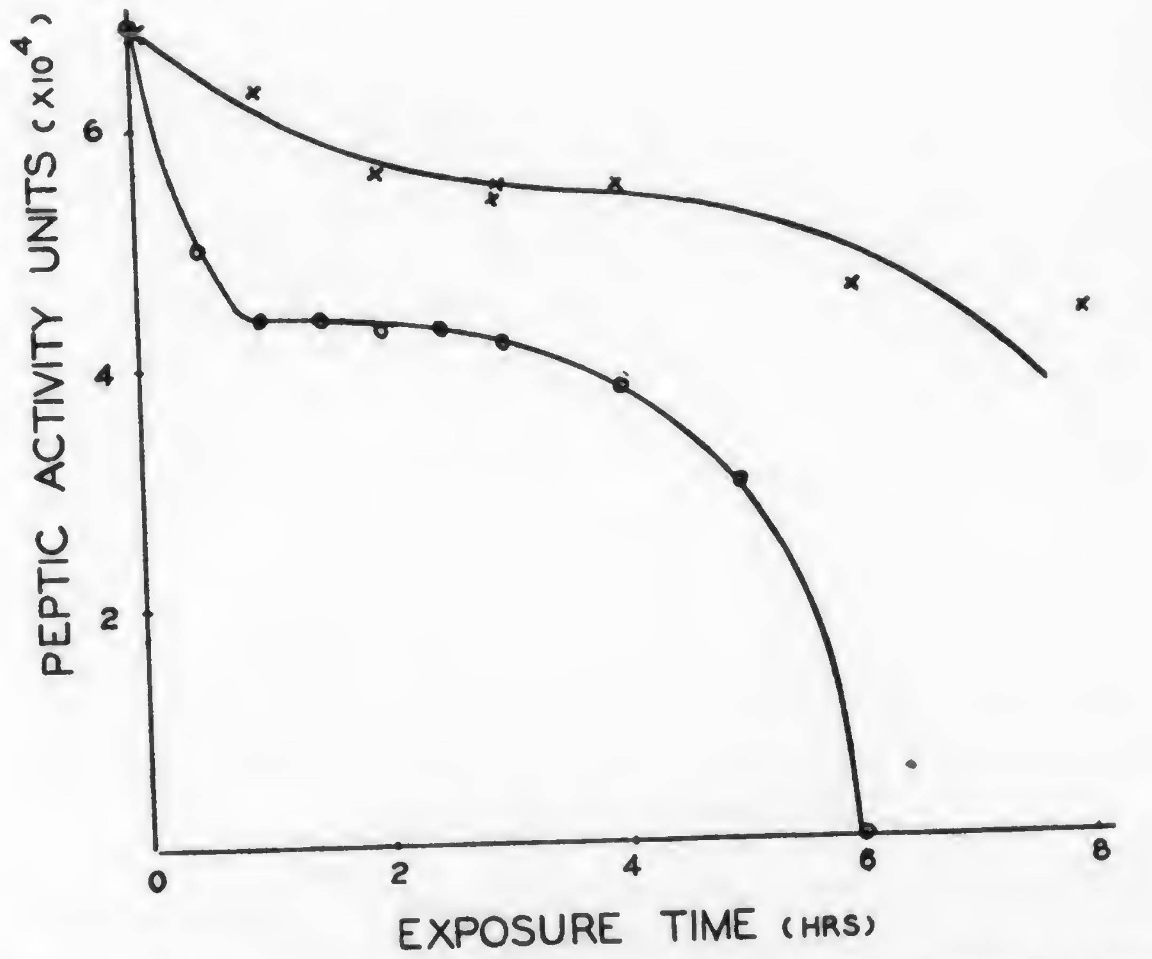


Fig. 3. Peptic activity *versus* exposure time at constant pressure. The upper curve is at 2500 kilos per sq.cm. and the lower at 4500 kilos per sq.cm.

The activity *versus* pressure curves at constant exposure times did not vary considerably in nature from one curve to another. The curve of Fig. 4 for 1 hour's exposure time was found to be similar to the one for an exposure time of 4 hours. On the basis of these results it can be said that peptic activity was relatively uninfluenced by the length of the exposure time interval. Fig. 5 for rennin was obtained at a constant exposure time of 1 hour. Qualitatively, the activity curve (inverse of the clotting time curve) was found to be similar in general nature to those of pepsin under like conditions.

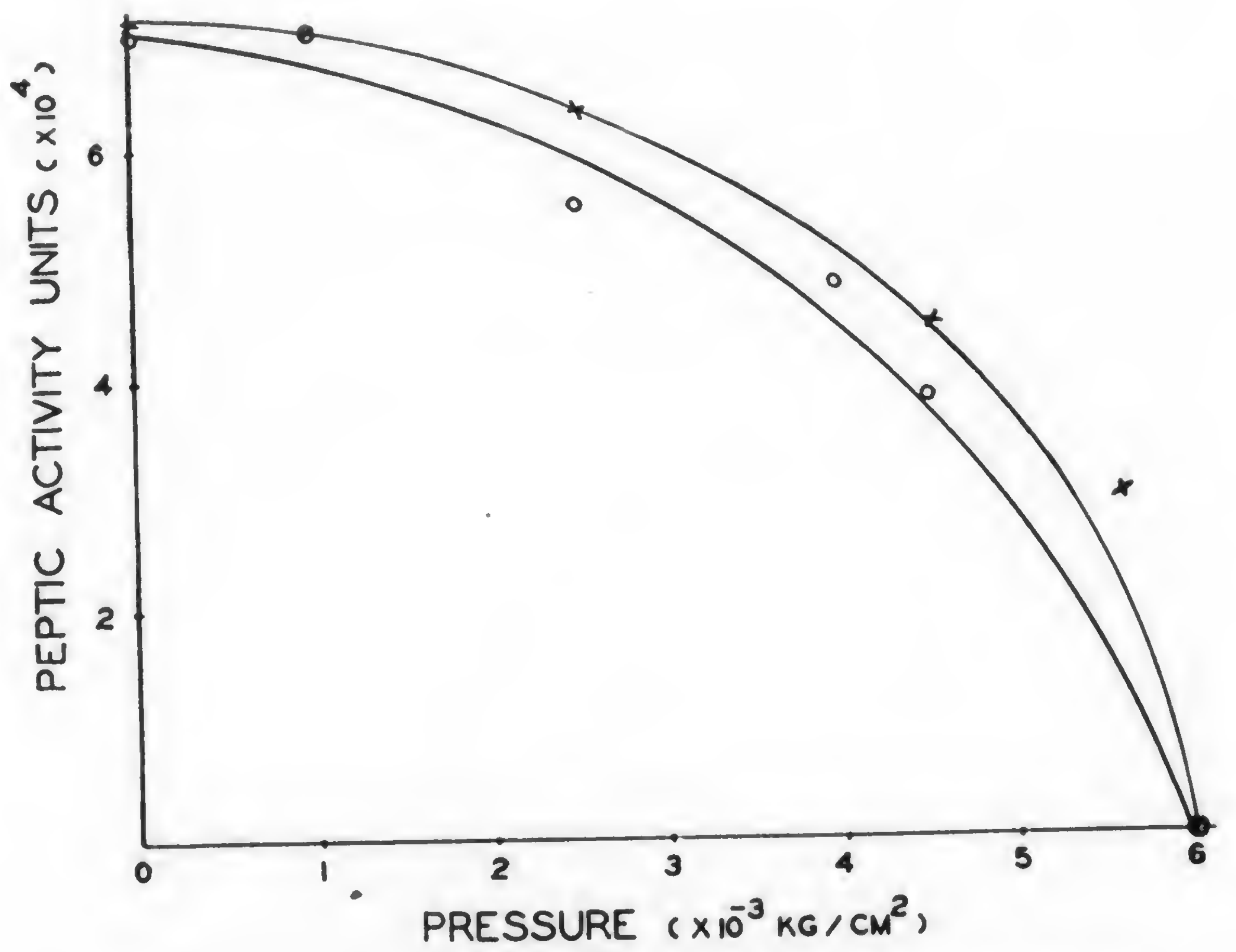


Fig. 4. Peptic activity *versus* pressure at constant exposure time. The upper curve is at 1 hour and the lower at 4 hours.

Several experiments were repeated in order to observe the reproducibility of data. There was no appreciable error due to control solutions of both pepsin and rennin, as in practically every case fresh solutions gave the same measured activities. Under the pressure conditions there was greater possibility of error, although the error in the measurement of pressure alone according to standard methods was considered to be negligible. As a measure of reproducibility, the peptic activity curve at 2500 kilos per sq.cm. of Fig. 3 has two values for an exposure time of 3 hours. It will be seen that the difference is about 0.2 of a peptic unit, a value that is commensurate with the reproducibility obtained in general. However, it must be emphasized that the intrinsic errors in the reactions were not subject to control and it is very likely that these errors were responsible for the deviations observed on several of the curves, namely those of Fig. 4.

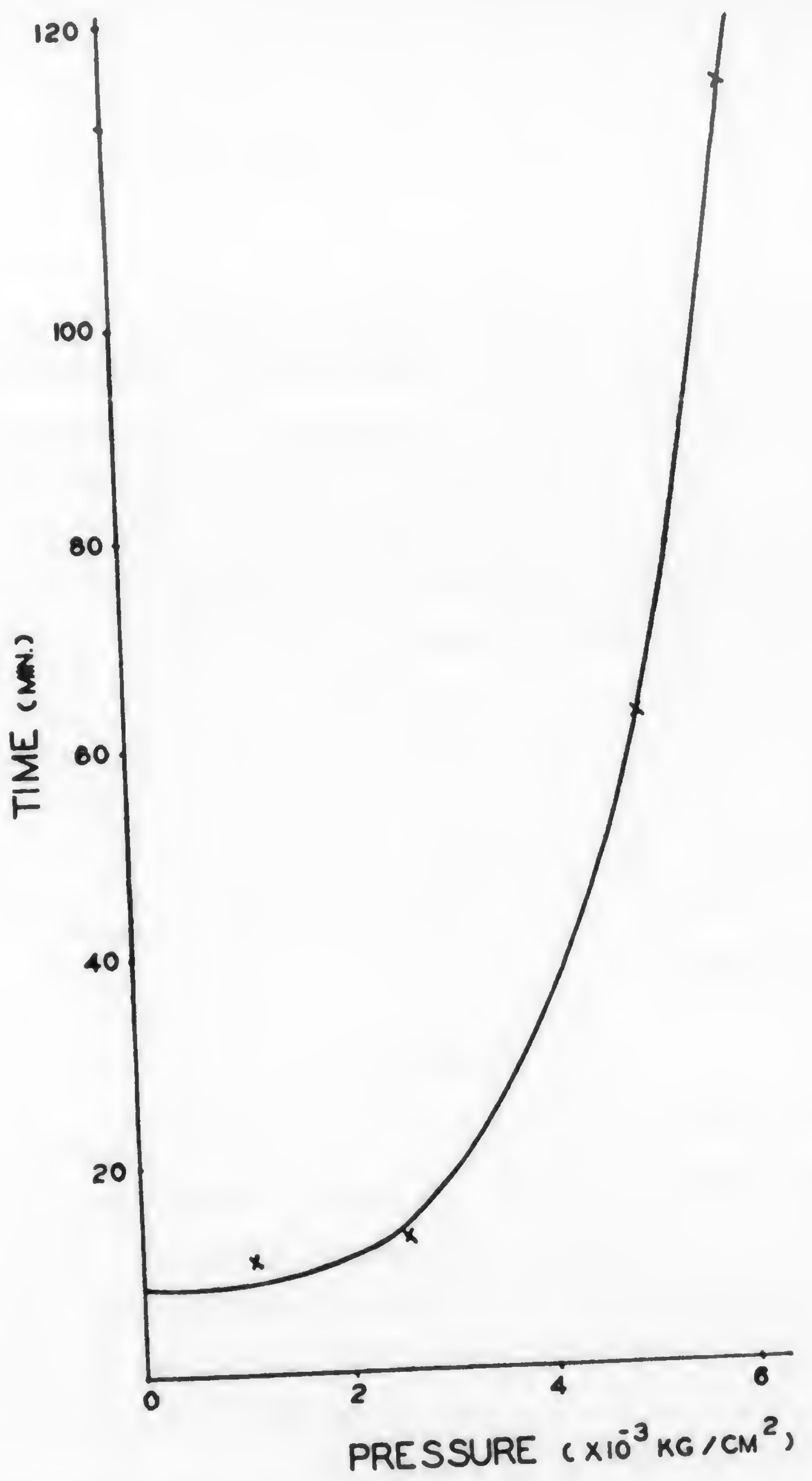


Fig. 5. Clotting time of rennin *versus* pressure at exposure times of 1 hour. The control clotted at 10 minutes in every case.

Hydrolysis under pressure might be expected to cause some of the complications that were observed. Amino nitrogen values

(Van Slyke) were measured for five samples of pepsin solution that had been treated with a pressure of 10,000 kilos per sq.cm. for 1 hour at 23°. No change of amino nitrogen content was found and it must be concluded that no appreciable hydrolysis resulted during the pressure treatment.

Although it is evident that these data show that the loss of activity of pepsin and rennin due to pressure is not a result of simple denaturation, the physical appearance of the denatured substance often was similar to that which had been denatured by heat. Both enzyme solutions were heavily coagulated by pressure treatment at 10,000 kilos per sq.cm., a result that appeared to be the same as when the solutions were heated to 100°.

Physically, denaturation by pressure is not similar to denaturation by heat. When a substance is heated at atmospheric pressure, energy is put into it and measured by the temperature rise. But when a substance is compressed isothermally, not only is work done externally but also internally by means of the attractive forces of the molecules. For example, in isothermal compression of the solutions used in this study to a pressure of 1000 kilos per sq.cm. it can be shown by thermodynamics that 7 times more energy flows out of the solutions than is put in by the work of compression. Energy, then, cannot be the sole factor for denaturation by pressure.

SUMMARY

The loss of activity of pepsin and rennin has been studied as a function of pressure and exposure time. Activity decreased with pressure increase at constant exposure time and was completely destroyed at pressures ranging between 5000 and 6000 kilos per sq.cm. Activity decreased also with increase of exposure time at constant pressure, but to a lesser degree. Pressure was important in determining the activity of these enzymes, but time of exposure was relatively unimportant. Loss of activity appeared to be dependent on certain buffers and the pH. A few observations showed that inactivation of pepsin and rennin by pressure depended strongly on the temperature.

It has been shown that denaturation by pressure was the probable cause for inactivation, although the reaction is not a simple monomolecular one. Pressure treatments as high as 10,000 kilos per sq.cm. caused no change in amino nitrogen content and it has been concluded that no hydrolysis was caused by pressure. The product of denaturation by pressure appeared similar to that which had been denatured by heat. It has been shown that the energy relations must be quite different in the two processes. Consequently, it can be said that energy changes are not alone responsible for denaturation and deactivation of the enzymes pepsin and rennin by pressure.

BIBLIOGRAPHY

1. Bridgman, P. W., Physics of high pressure, New York (1931).
2. Cohen, E., and Schut, W., Piezochemie kondensierter Systeme, Leipzig (1919); Cohen, E., Physicochemical metamorphosis and some problems in piezochemistry, New York (1928).
3. Bridgman, P. W., *J. Biol. Chem.*, **19**, 511 (1914).
4. Bridgman, P. W., and Conant, J. B., *Proc. Nat. Acad. Sc.*, **15**, 680 (1929).
5. Basset, J., and Macheboeuf, M. A., *Compt. rend. Acad.*, **195**, 1431 (1932).
6. Basset, J., Macheboeuf, M. A., and Sandor, G., *Compt. rend. Acad.*, **197**, 796 (1933).
7. Basset, J., Lisbonne, M., and Macheboeuf, M. A., *Compt. rend. Acad.*, **196**, 1540 (1933).
8. Basset, J., and Macheboeuf, M. A., *Compt. rend. Acad.*, **196**, 67 (1933).
9. Basset, J., Wollman, E., Macheboeuf, M. A., and Bardach, M., *Compt. rend Acad.*, **196**, 1138 (1933).
10. Frankel, S., and Meldolesi, G., *Biochem. Z.*, **115**, 85 (1921).
11. Hite, B. H., Giddings, N. J., and Weakley, C. E., Jr., *West Virginia Exp. Stat., Bull. 638* (1902); *Bull. 145* (1914).
12. Larson, W. P., Hartzell, T. B., and Diehl, H. S., *J. Infect. Dis.*, **22**, 271 (1918).
13. Dow, R. B., and Matthews, J. E., Jr., *Phil. Mag.*, **27**, 637 (1939).
14. Northrop, J. H., *J. Gen. Physiol.*, **16**, 41 (1932-33).
15. Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **16**, 59 (1932-33).
16. Tauber, H., and Kleiner, I. S., *J. Biol. Chem.*, **96**, 745 (1932).
17. Stone, J. B., and Alsberg, C. L., *J. Biol. Chem.*, **78**, 557 (1928).

BULLETIN 400 SEPTEMBER 1940

Deposition and Retention of Sprays on Apples. II.

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

CONTENTS

PAGE

Purpose and Plan of Experiment 1

Experimental Methods 1

Sampling—Location of apple trees and varieties used—Analytical methods—Climatic conditions

Experimental results 4

1937 experiments—1938 experiments

Discussion 16

Ratio of lead to arsenic in spray deposits—Timing of spray applications — Spreading and adhesive agents—"Dynamite" sprays—Codling moth control —Spray injury

Summary 21

Bibliography 22

Deposition and Retention of Sprays on Apples. II.*

DONALD E. H. FREAR and HARLAN N. WORTHLEY†

IN an earlier publication *(4)* the authors presented data on the amounts of lead and arsenic deposited and retained upon the fruits and foliage of two varieties of apples when sprayed with various combinations of insecticides and fungicides applied according to different schedules. The results were obtained during the growing seasons of 1934 and 1935.

The introduction of new spray materials and mixtures, such as the so-called "dynamite" sprays *(8 and 9)*, has made further investigations of a similar nature necessary in order to compare the new fungicides and insecticides with the accepted materials and methods. Further, as in all field experiments, it was felt that the conclusions reached in the earlier work should be tested, in order to eliminate seasonal variations in so far as possible.

PURPOSE AND PLAN OF EXPERIMENT

The studies were conducted in a manner similar to those reported earlier. Various combinations of spray materials were applied to apple trees, and samples of fruit were collected during the growing season, usually before and after each spray application, and then at intervals until the fruits were harvested. The fruits in each sample were then analyzed for spray deposits. In most cases, fruits were picked from both the upper and lower branches of the trees, and analyzed separately, in order further to study spray distribution on the trees. All sprays used contained lead arsenate in combination with other materials, and the results reported deal only with the deposition and retention of lead and arsenic.

EXPERIMENTAL METHODS

Sampling.—Samples of 20 apples each were taken at random both from the bottoms and the tops of the trees. Fruits from several trees were collected and combined for each sample. At harvest time, the size of the fruits was such that smaller samples were necessary, in which case duplicate weighed samples of 700 grams, three to six apples, were taken from each treatment. Samples following a spray application were taken immediately after the spray had dried, and all samples were picked and handled with great care, to minimize loss of a mechanical nature.

---

*Authorized for publication August 13, 1940.

†Assistant Professor of Agricultural and Biological Chemistry and Professor of Economic Entomology, respectively. This investigation was conducted jointly by the departments which these two workers represent.

Location of apple trees and varieties used.—Orchard plots laid out as randomized single tree replicates by the department of zoology and entomology for comparison of spray treatments in codling moth control supplied the fruit samples. During 1937, mature trees of the Jonathan variety, in the orchards of E. H. Sachs, Biglerville, Pa., were used for the spraying experiments. Six trees were sprayed in the same manner, as randomized, single-tree replicates, in each of eight different treatments, as will be described later.

In 1938, mature Stayman Winesap and Jonathan trees were used, located in the orchard of L. W. Garrettson, Arendtsville, Pa. As in the previous year, each treatment was replicated six times, and there were seven treatments.

Analytical methods.—The fruits were removed to the laboratory as soon as possible after picking and washed individually in a boiling solution containing 2 per cent hydrochloric acid by weight. The washings from a given sample were combined and made to a definite volume, 500 ml. Portions of this wash solution were then digested and analyzed for lead by the method of Frear and Haley *(3)* and for arsenic by the bromate distillation method *(1)*. This method for the determination of arsenic has been found to be considerably more reliable than the Gutzeit method used in the previous study.

Measurements of the transverse and vertical diameters of the apples were made immediately after washing, and from these, using the average diameter in the formula for the surface area of a sphere, the surface areas of the apples were calculated.

Climatic conditions.—Since climatic conditions are a factor in the effectiveness of any orchard spray application, monthly mean temperatures and precipitation are given for the growing seasons of 1937 and 1938 at Arendtsville, table 1. It is to be noted that in 1937 the mean temperatures were very close to the 16-year average, being slightly above the average during May, June, July, and August, but slightly below the average during September and October. During 1938 the temperatures were even closer to the average; the only month during the period studied which showed any considerable divergence from the average being August, when the mean temperature was approximately 3 degrees above the 16-year average.

Table 1.—Mean temperatures and rainfall at Arendtsville, Pa., 1937 and 1938.

MONTHS	MEAN TEMPERATURE			RAINFALL		
	1937	1938	Normal	1937	1938	Normal
	Degrees	*Degrees*	*Degrees*	*Inches*	*Inches*	*Inches*
May	62.4	60.2	60.1	2.76	5.76	3.84
June	72.8	69.4	70.5	5.33	2.67	5.09
July	75.6	75.4	74.5	5.99	2.94	3.88
August	75.0	75.6	72.4	5.50	8.53	4.75
September	63.0	63.4	65.9	3.04	2.49	3.66
October	50.6	55.5	54.2	8.96	1.70	3.50

Rainfall during 1937 was greater than the average, although during May there was a considerable deficiency. During July and October, however, there was an excess. In 1938 there was less rainfall than usual, with the exception of the month of August, when nearly twice the normal amount fell.

Because distribution of rainfall as well as monthly totals is important, daily precipitation is shown, fig. 1. During the period under consideration, May 15 to September 22 in 1937, measurable amounts of rain fell on 50 days and the total rainfall during the period was 20.60 inches. On 12 separate days during this period, rainfall of more than 0.5 inch was reported.

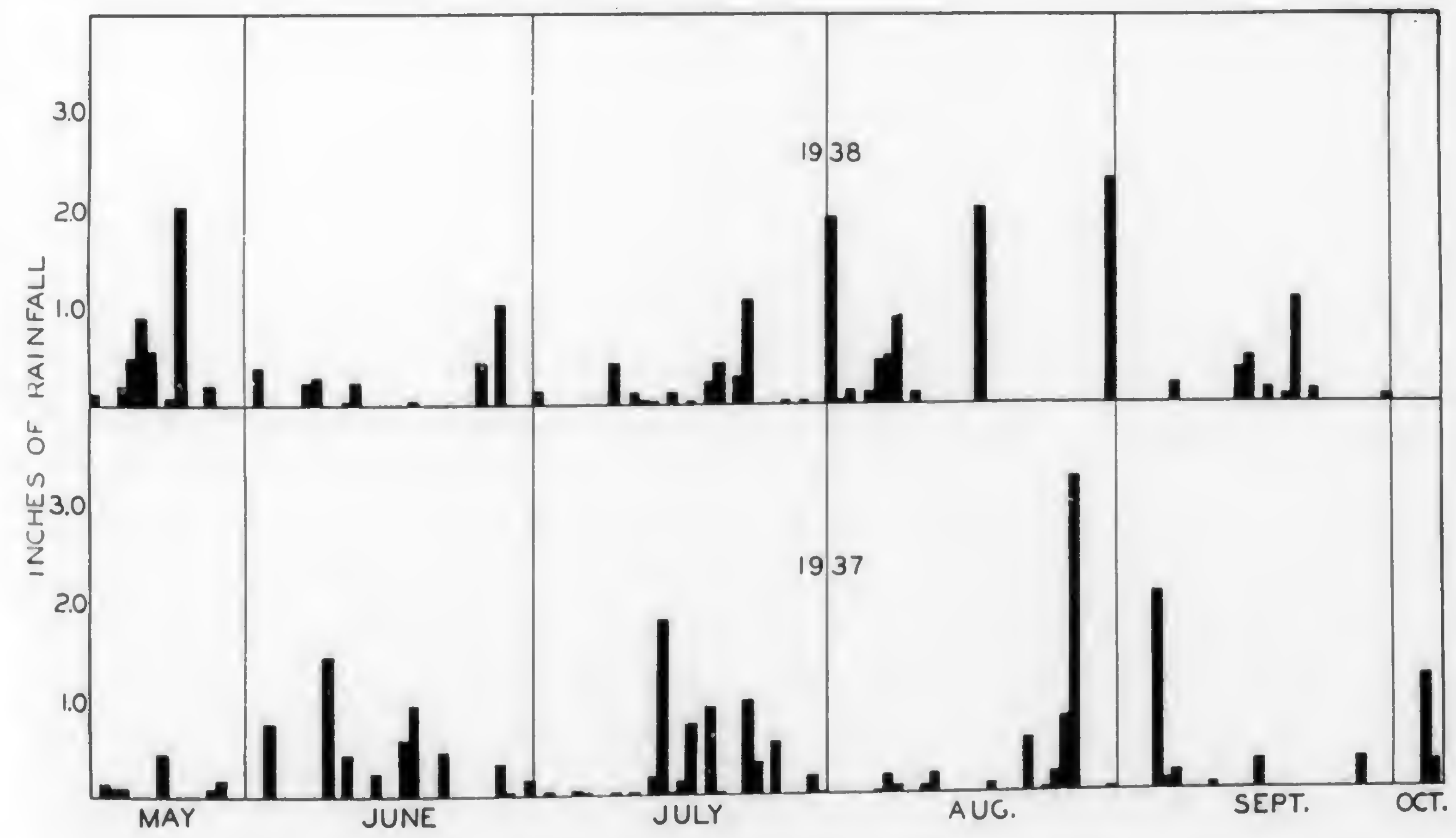


Fig. 1.—Distribution of rainfall during the 1937 and 1938 growing seasons at Arendtsville, Pa.

During the season of 1938, in the period between May 15 and October 5, measurable amounts of rain fell on 47 separate days, with a total rainfall of 23 inches. On 10 separate days during the period, more than 0.5 inch of rain fell.

From these records it would appear that the two seasons were quite comparable as far as amounts and distribution of rainfall were concerned.

EXPERIMENTAL RESULTS

1937 experiments.—The materials applied in the several treatments used in 1937, and the times of application are given in table 2. Eight treatments, using in all six spray mixtures, were studied. Three schedules of applications were employed: (A) a 5-cover spray schedule, with sprays applied at intervals of 5, 14, 27, and 44 days after the first application; (B) a 5-cover spray schedule with sprays applied at intervals of 11, 22, 33, and 44 days after the first application; and (C) a 3-cover spray schedule with sprays applied at intervals of 11 and 22 days after the first application. The first cover spray was applied at the same date, June 4, in all treatments.

Table 2.—Constituents and times of application of spray mixtures used in the 1937 experiments.

TREATMENT NUMBERS	AMOUNTS PER 100 GALLONS[*]	DATES OF APPLICATION							
		6-4	6-10	6-15	6-18	6-26	7-1	7-7	7-17
1	Lead arsenate, 3 lbs.	x[†]	x		x		x		x
	Copper Hydro 40, 2 lbs.	x	x						
	Fish oil, 1 pt.	x	x		x		x		x
2	Lead arsenate, 3 lbs.	x	x		x		x		x
	Flotation sulfur paste, 10 lbs.	x	x						
	Fish oil, 1 pt.	x	x		x		x		x
3	Lead arsenate. 3 lbs.	x		x		x		x	x
	Flotation sulfur paste, 10 lbs.	x		x					
	Fish oil, 1 pt.	x		x		x		x	x
4	Lead arsenate, 3 lbs.	x		x		x		x	x
	Copper Hydro 40, 2 lbs.	x		x					
	Fish oil, 1 pt.	x		x		x		x	x
5	Lead arsenate, 3 lbs.	x		x		x			
	Copper Hydro 40, 2 lbs.	x		x					
	Fish oil, 1 pt.	x							
	Petrocide, 2 qts.			x		x			
6	Lead arsenate, 3 lbs.	x		x		x			
	Copper Hydro 40, 2 lbs.	x		x					
	Summer petroleum oil + oleic acid (19:1), 2 qts.			x		x			
	Water + triethanolamine (6:1), 8 fl. oz.			x		x			
7	Lead arsenate, 3 lbs.	x		x		x		x	x
	Copper Hydro 40, 2 lbs.	x		x					
	Grasselli S-S, 2 fl. oz.	x		x		x		x	x
8	Lead arsenate, 3 lbs.	x		x		x		x	x
	Copper Hydro 40, 2 lbs.	x		x					
	Soybean flour, 1 lb.	x		x		x		x	x

[*] 12 gallons of spray per tree per application, except in treatments 5 and 6 where in the second and third covers, the amount was increased to 25 gallons per tree.

[†] x indicates application of material at time indicated.

Treatments 1 and 4 were identical with respect to the amounts of ingredients applied, the difference being in the times of application. The same was true of treatments 2 and 3. The latter pair of treatments, 2 and 3, differed from the former, 1 and 4, only in the fungicide applied, a commercial copper fungicide, "Copper Hydro 40", being used in treatments 1 and 4, while flotation sulfur was used in treatments 2 and 3. The comparison between two otherwise identical sprays, one applied at regular intervals and the other at intervals increasing in length with the rate of growth of the apple fruits, was made in order to secure further information on a point raised in the preceding publication *(4)*. In previous work it was noted that the surface area of apple fruits did not increase at a regular rate during the growing season, but doubled in extent rapidly (in approximately six days) during the early period of growth, with a progressively less rapid rate of increment as the season advanced. It was reasoned, therefore, that the early spray applications, to be most effective, should be applied at short intervals, with progressively longer intervals between spray applications as the season advanced. This so-called "growth schedule" of spray applications was, therefore, proposed and tried in a preliminary way during the season of 1935. The experiments of 1937 were designed to give further evidence on the relative deposit and retention of sprays applied in this way, as compared to the conventional method of timing spray applications at equal intervals.

Treatments 5 and 6 were included to determine the deposition and retention of lead arsenate from the so-called "dynamite" or inverted spray mixtures proposed first by the workers at the Washington Agricultural Experiment Station *(8)*. In these mixtures the lead arsenate is preferentially wetted by the oil used and adheres to the plant surfaces, so that it is possible to build up deposits by continued spraying. This is a distinctly different situation than that which obtains when the usual spray mixtures are applied; with the latter, spraying continued after complete wetting of the plant surfaces results only in "run off" of the spray mixture, and wasted materials.

Treatment 6 consisted of three cover sprays, in two of which a mixture composed of: (a) Lead arsenate, 3 pounds; (b) "Copper Hydro 40," 2 pounds; (c) A mixture of 19 parts summer petroleum oil plus 1 part oleic acid, 2 quarts, and (d) A mixture of 6 parts water plus 1 part triethenolamine, 8 fluid ounces, was used. Treatment 5, in the first cover spray application, consisted of the same mixture used in treatments 1 and 4, but in the second and third cover sprays, 2 quarts of "Petrocide", a proprietary material designed to produce a deposit similar to that from the "dynamite" mixtures, were added. Double quantities of spray were applied in the two "dynamite" applications in treatments 5 and 6.

Treatments 7 and 8 were included to study the effect of two adhesive agents on the deposition and retention of lead arsenate. In treatment 7, 2 fluid ounces of "Grasselli Spreader-Sticker" replaced the fish oil used in treatments 1 and 4, while 1 pound of soybean flour was substituted for the fish oil in treatment 8. Otherwise the mixtures applied and schedule of application were the same as in treatment 4.

The results of the analyses of the apple fruit samples during 1937 are shown in figure 2, including both lead and arsenic, in milligrams per square meter of fruit surface, for samples taken from both the tops and the bottoms of the trees. Lead deposits are shown by solid lines; arsenic (as As_2O_3), by dotted lines.

As in the previous work, the deposits of both lead and arsenic on the bottoms of the trees was greater than on the tops. The deposits on the tops and bottoms of the trees in treatment 7 were almost the same, this being the only exception. The extent of this difference apparently depends on the thoroughness of the original application. The deposits of lead were, as before, considerably higher than the deposits of arsenic, although there was considerable variation in the lead: arsenic ratio. This will be discussed in more detail later.

Perhaps the most significant fact observed in this comparison of materials and schedules is the high deposit after the second application of the "dynamite" sprays, treatments 5 and 6. It appears that the continued spraying in treatment 6 gave a "build up" greater than from any other spray application on the same day, although all spray mixtures contained the same amounts of lead arsenate. This is shown in table 3, in which is revealed the difference between the deposits on samples taken immediately before and immediately after spray treatment.

Table 3.—Comparison of lead and arsenic deposited upon apple surfaces in different parts of trees in spray application of June 26, 1937.

TREATMENT NUMBERS	Pb		As_2O_3	
	Top	Bottom	Top	Bottom
	Mg./M²	*Mg./M²*	*Mg./M²*	*Mg./M²*
Mean of treatments 3, 4, 7, and 8*	126.4	159.9	45.0	52.1
5	120.5	150.4	71.2	69.8
6	147.5	235.2	55.9	114.5

*Treatments 1 and 2 were not applied on this date.

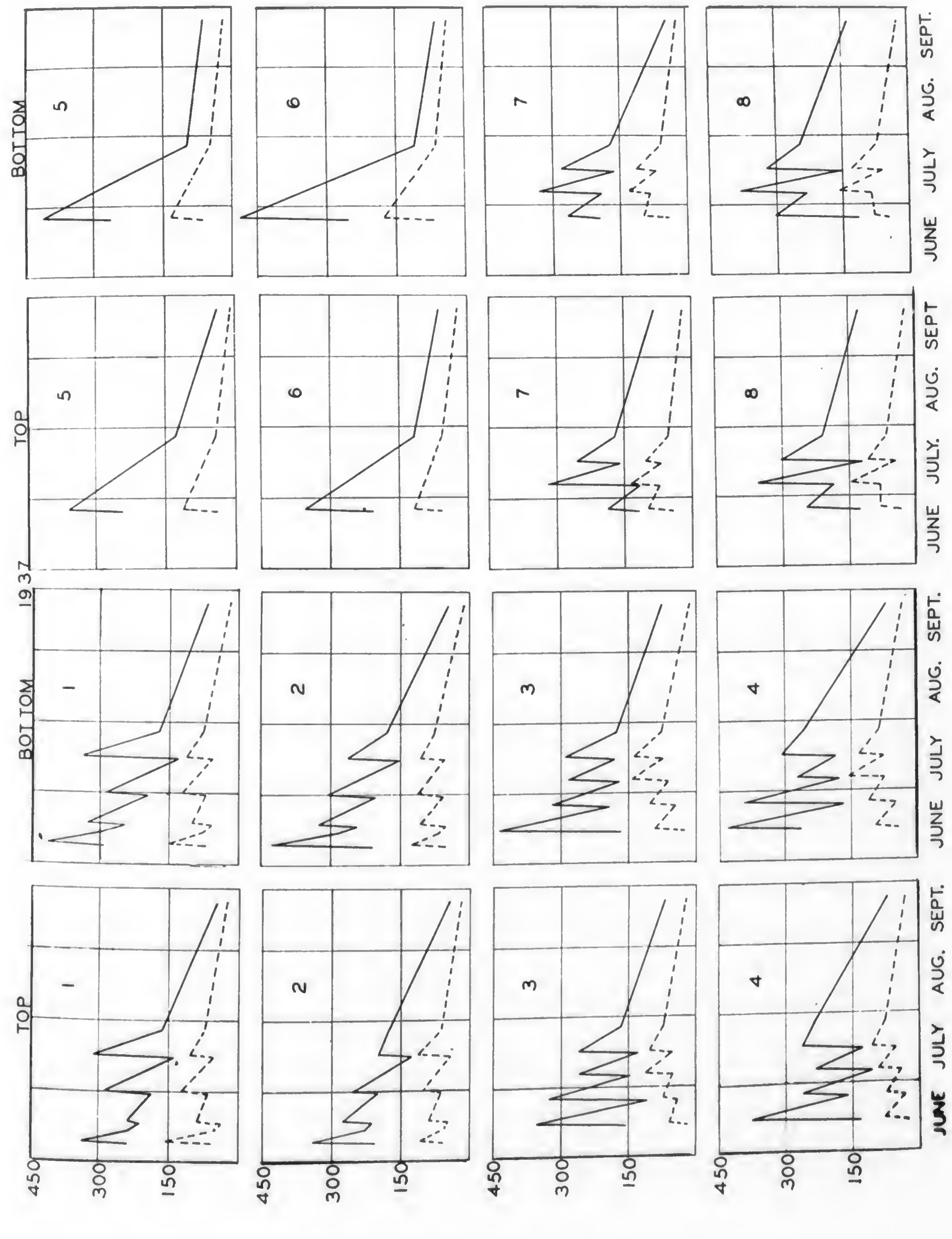


Fig. 2.—Lead and arsenic (as As_2O_3) on apple fruits, 1937 season. The solid lines represent lead, the dotted lines arsenic in milligrams per square meter.

Note the differences between the samples taken from the tops and bottoms of the trees: Unless great care is taken to cover the tops of the trees completely, this portion of the tree is likely to receive inadequate coverage. Note also the high deposit produced by the "dynamite" mixture applied on June 26 to treatments 5 and 6. The slope of the lines from late July to harvest shows that the deposits on these two treatments were lost less rapidly during this period.

For purposes of comparison of the effectiveness of the deposit in terms of protection, it has been found that charts showing the *average amount of spray deposit by periods* during the growing season are quite satisfactory. Such charts are shown in figure 3, giving the average deposits of lead in milligrams per square meter of fruit surface for both the tops and bottoms of trees during the period of sampling. The arsenic figures closely parallel those for lead and are, consequently, omitted from the charts.

Comparing treatment 1, in which the sprays were applied according to the "growth schedule", with treatment 4, applied at regular intervals of 11 days, but otherwise identical, little difference in deposit can be noted. It would appear that in treatment 1, the close spacing of the early sprays resulted in a higher deposit (approximately 360 milligrams per square meter) than in treatment 4 (approximately 300 milligrams per square meter) during the first few days after the original application. This is offset, however, by the higher average deposit in treatment 4 later in the season, between the last application and harvest.

Comparisons of treatments 2 and 3 show much the same condition, although the average deposit present during the period between the last spray application and harvest shows no significant difference in this case. Contrary to expectation, spacing of the spray applications to compensate for the rapid early development of the apple surfaces did not result in the maintenance of a consistently higher level of lead deposit than the spacing of applications at equal intervals. There appeared to be no significant difference between the deposits produced by the mixtures containing flotation sulfur, 2 and 3, and those containing "Copper Hydro 40," 1 and 4.

Treatments 7 and 8, differing from treatment 4 only in the adhesive agents used, showed almost the same average deposit of lead during the period studied as treatment 4. The superior adhesiveness of the mixture containing soybean flour is shown by the high average level of lead maintained during the period from July 28 to harvest.

With treatments 5 and 6, the deposits of lead were at a relatively high level for a surprisingly long period, at least from June 25 to July 28, when samples were taken. The average deposits of lead on fruits during this period in treatment 5 were 245 milligrams per square meter at the tops of the trees, and 255 at the bottoms; while in treatment 6 during the same period the average deposits at the tops of the trees were 235 milligrams per square meter and at the bottoms almost 300. This is particularly noteworthy since these

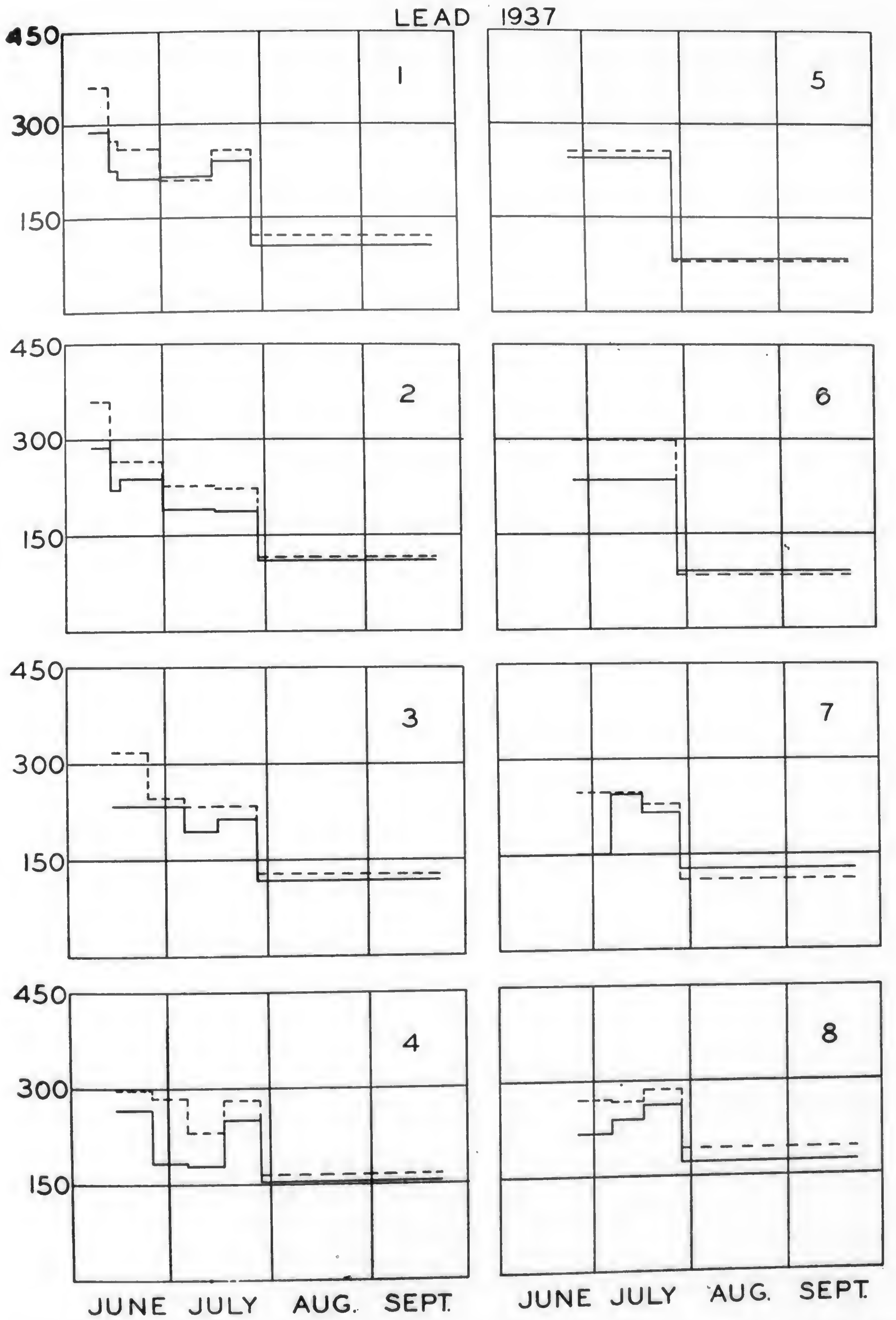


Fig. 3.—Average deposit of lead on apple surfaces in milligrams per square meter during periods between sampling, 1937. Dotted lines represent samples from bottoms of trees, solid lines samples from tops of trees.

trees received only three cover sprays, of which the last two contained the inverted or "dynamite" mixture. At the end of this period, treatments 5 and 6 showed the effects of an additional three weeks of weathering, although the amount of arsenic remaining in treatment 6 compared favorably with that in treatments receiving more extended spray schedules, table 4.

Table 4.—Lead and arsenic deposits on Jonathan apples under experimental spray treatment, July 28, 1937.

TREATMENT NUMBERS	DAYS FROM LAST APPLICATION	Pb	As_2O_3
		$Mg./M^2$	$Mg./M^2$
1	11	169	73
2	11	178	71
3	11	171	73
4	11	248	83
7	11	173	55
8	11	234	70
5	32	113	44
6	32	113	60

There were apparently few significant differences in the retention of the deposits among the different treatments receiving five cover sprays, with the exception of treatment 8, in which soybean flour was used as a supplement. This treatment showed significantly higher retention of lead at harvest, table 5. In addition, treatments 1 and 2 appear to have retained slightly less lead, particularly at the tops of the trees, than did the other 5-application treatments, although the figures are possibly of slight significance. However, it appears significant that treatment 6, receiving only three cover sprays, had a deposit of both arsenic and lead at harvest approximately as great as any of the 5-cover spray treatments except treatment 8, while treatment 5, in general, had considerably less.

Table 5.—Lead and arsenic deposits at harvest, September 22,1937.

TREATMENT NUMBERS	Pb		As_2O_3	
	Top	Bottom	Top	Bottom
	$Mg./M^2$	$Mg./M^2$	$Mg./M^2$	$Mg./M^2$
1	45.3	67.8	22.1	16.3
2	45.6	47.7	16.7	12.4
3	68.9	76.8	20.8	14.9
4	73.0	72.7	31.9	31.1
5*	37.8	63.8	8.8	18.5
6*	63.8	61.4	21.7	36.1
7	80.4	44.2	18.4	19.5
8	125.3	139.1	21.9	24.4

*Three spray applications: all other treatments received five applications

1938 experiments.—The materials applied in the eight treatments used in 1938, together with the times of application, are given in table 6. These treatments were designed to give further information on certain points raised by the analysis of the work of the previous year, as well as to continue studies previously undertaken. Treatments 3 and 4, for example, were identical in composition, but, while in treatment 3 the applications were made at regular intervals of 11 days following the first spray, the trees

Table 6.—Constituents and times of application of spray mixtures used in the 1938 experiments.

TREATMENT NUMBER	AMOUNTS PER 100 GALLONS	DATES OF APPLICATION									
		5-17	5-25	5-31	6-6	6-9	6-14	6-17	6-30	8-8	8-20
1	Lead arsenate, 3 lbs.	x*		x			x		x		
	Lime-sulfur, 1.3 gals.	x		x					x		
	Skimmilk powder, 2 lbs.	x		x			x		x		
2	Lead arsenate, 3 lbs.		x		x			x	x		
	Lime-sulfur, 1.3 gals.		x		x				x		
	Skimmilk powder, .5 lb.		x		x			x	x		
	Hydrated lime, .5 lb.		x		x			x	x		
3	Lead arsenate, 3 lbs.		x		x			x	x		
	Lime-sulfur, 1.3 gals.		x		x				x		
	Soybean flour, 1 lb.		x		x			x	x		
	Hydrated lime, .5 lb.		x		x			x	x		
4	Lead arsenate, 3 lbs.		x	x		x					
	Lime-sulfur, 1.3 gals.		x	x		x					
	Soybean flour, 1 lb.		x	x		x					
	Hydrated lime, .5 lb.		x	x		x					
5	Lead arsenate, 3† lbs.		x	x†		x					
	Copper phosphate mix‡, 16 lbs.		x								
	Oil + oleic acid mix§, 2 qts.			x		x					
	Water + monoethanolamine (7:1), 8 fl. oz.			x		x					
7	Lead arsenate, 3† lbs.		x		x†						
	Copper phosphate mix‡, 16 lbs.		x								
	Oil + oleic acid mix§, 2 qts.				x						
	Water + monoethanolamine (7:1), 8 fl. oz.				x						
	Black leaf 155, 8 lbs.								x		
8	Lead arsenate, 3 lbs.		x		x						
	Lime-sulfur, 1.3 gals.		x		x						
	Soybean flour, 1 lb.		x		x						
	Hydrated lime, .5 lb.		x		x						
	Black leaf 155, 4[1] lbs.							x[1]	x[1]	x	x
	Orthol-K-Ready mix, 2 qts.									x	x

*x indicates application of material at time indicated.

†Double quantity (6 lbs.) at time indicated, and double dosage per tree.

‡Copper phosphate mix = 4 lbs. copper phosphate, 8 lbs. hydrated lime, 4 lbs. bentonite.

§Oil + oleic acid mix = (A) 3 parts kerosene + 1 part fish oil, (B) oleic acid; combined in the final mix in the ratio of 19 parts (A) to 1 part (B).

[1] Double quantity (8 lbs.) used at time indicated.

receiving treatment 4 were sprayed on the sixth and fifteenth days following the first application, according to the "growth schedule". Treatment 4 received three spray applications, while treatment 3 received four.

Treatments 1 and 2 were identical in composition and number of applications, but the sprays applied in treatment 1 were timed according to the spray service recommendations. In treatment 2 the applications were timed by means of bait-trap records of codling moth flight, and were applied when it became evident that large numbers of eggs were present. Skimmilk powder used as a sticker in treatment 2 was compared with soybean flour used in treatment 3.

The foregoing treatments were compared with abbreviated schedules in which high deposits of lead arsenate were sought by doubling the concentration of this constituent and the dosage per tree in one of three spray applications, treatment 5, or in one of two applications, treatment 7[1], both treatments using "modified W.S.C. dynamite" mixtures *(9)*. Finally, treatment 8, using a mixture similar in composition to that used in treatments 3 and 4, received two applications of this mixture at the same times as treatment 7, followed later in the season by four applications of nicotine bentonite and summer oil.

It is to be noted that before the inverted mixtures were applied in treatments 5 and 7, copper phosphate was used as the fungicide, applied in a mixture containing lime and bentonite, while in all other treatments lime-sulfur solution was used.

The results of the chemical analyses made on the Stayman samples collected during the 1938 season, fig. 4, are calculated as milligrams of lead (solid line) and arsenic (as As_2O_3—dotted line) per square meter of fruit surface. The deposits of both lead and arsenic were considerably greater at the bottoms than at the tops of the trees in all treatments except 4. The difference between top and bottom deposits in 1938 was greater than in the previous year. This may be attributed to the fact that the 1937 operator had been especially trained to concentrate on the tops of the trees in spraying, and illustrates the necessity for emphasis upon this point.

As in 1937, the inverted or "dynamite" sprays produced significantly greater deposits per application than the ordinary mixtures used, regardless of the adhesive agent present in the latter. This was particularly true of the samples from the bottoms of the trees

---

[1] Treatment 6, not included in this discussion, was identical with treatment 7 except that it did not receive the supplementary application of Black Leaf 155 on June 30.

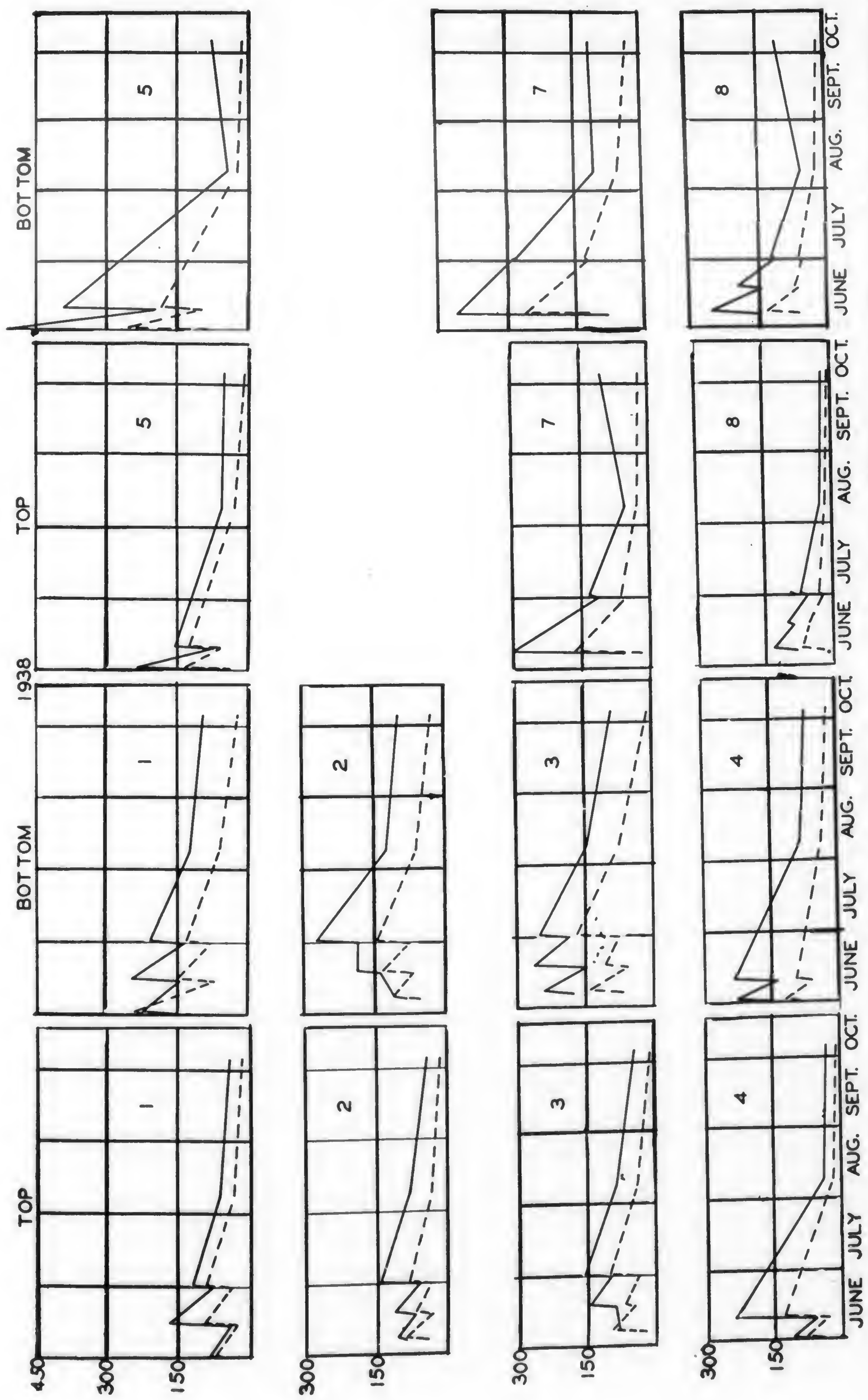


Fig. 4.—Lead, Pb, and arsenic (as As_2O_3) on Stayman apple fruits, 1938 season. The solid lines represent lead, the dotted lines arsenic in milligrams per square meter.

receiving treatments 5 and 7. Treatment 4, applied according to the "growth schedule", built up a relatively high deposit during the early part of the season, but the three applications did not maintain the deposit over a sufficiently long period for protection against later infestation.

In figure 5 the average deposits of lead between sampling dates are shown. It is even more apparent from this figure that the average deposits in treatments 5 and 7 were decidedly higher than from any other treatment during the period of first brood codling moth attack.

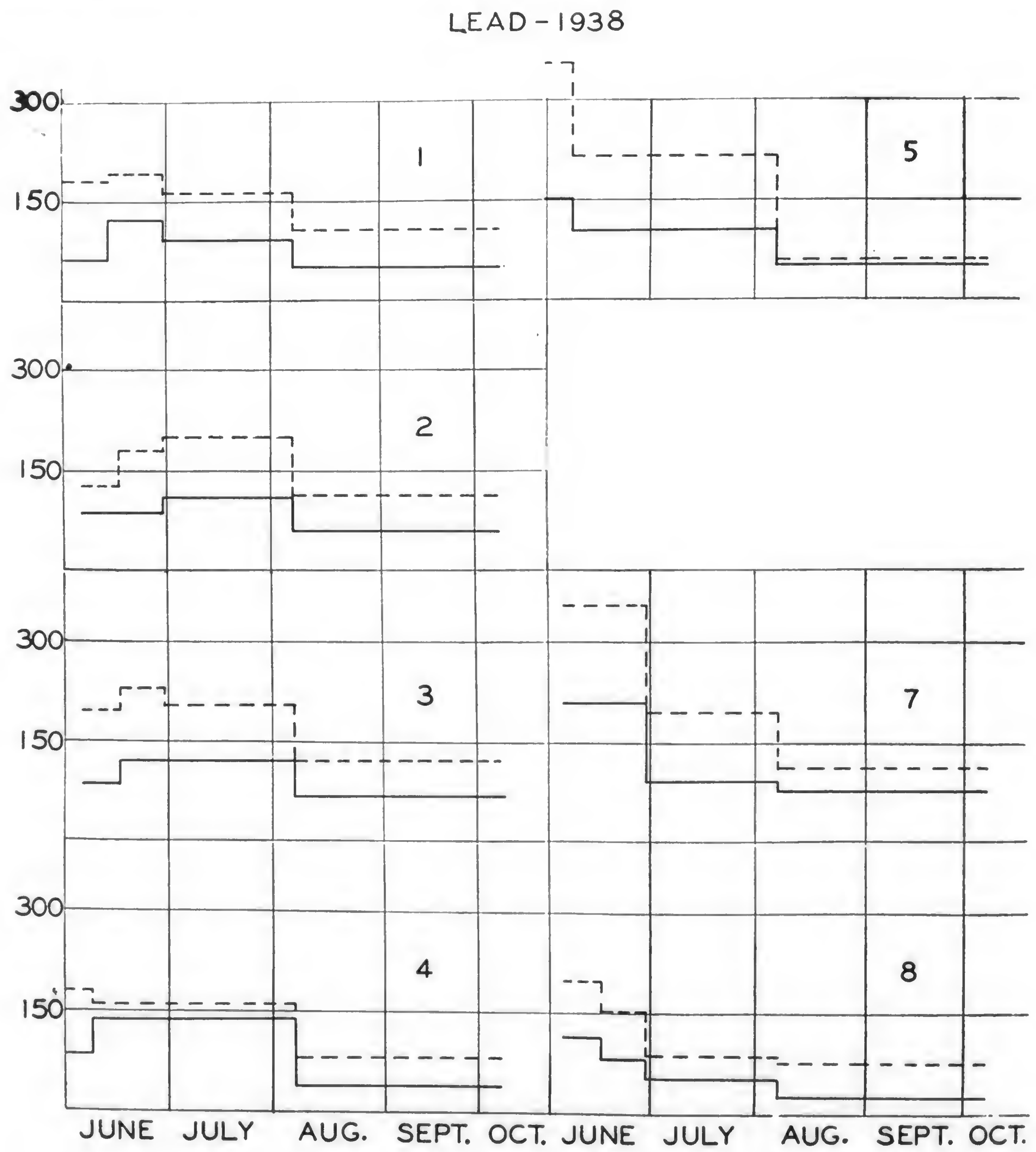


Fig. 5.—Average deposit of lead on apple surfaces in milligrams per square meter during periods between sampling, 1938. Dotted lines represent samples from bottoms of trees, solid lines samples from tops of trees.

As far as retention of deposits is concerned, it appears from figure 4 that the high deposits in treatments 5 and 7 decreased rather rapidly, as compared with those including later cover sprays. This was to be expected due to the rapid growth of the fruit subsequent to the last spray application. No significant differences appeared between the deposits from the mixtures containing skimmilk powder and those containing soybean flour.

At harvest, some rather interesting differences appeared in the amounts of the deposits. Apparently, the samples taken at this time were not sufficiently large, so that the sampling error is considerable. In treatments 5, 7, and 8, there is an apparent increase in lead deposit after the final spray applications were made, or between August 8 and harvest. Since most of these apparent increases were found on samples from the bottoms of the trees, it is possible that the action of rain in washing deposits from the upper to the lower portions of the tree was responsible; this does not account, however, for the apparent increase noted in the samples taken from the tops of trees in treatment 7.

The amounts of lead and arsenic on the fruit samples at harvest are given in table 7. The figures indicate that the deposits remaining on the fruits at the end of the season were similar in amount, probably because of the relatively long period of time during which the deposits were exposed to weathering. The amounts of lead on the fruits from trees receiving treatments 7 and 8 are somewhat higher than on those receiving the other treatments, possibly because of protection against weathering afforded by the later spray applications of nicotine-bentonite, plus oil in treatment 8.

Table 7.—Lead and arsenic deposits at harvest, October 5, 1938.

TREATMENT NUMBERS	Pb		As_2O_3	
	Top	Bottom	Top	Bottom
	Mg./M²	*Mg./M²*	*Mg./M²*	*Mg./M²*
1	42.38	95.12	16.38	21.79
2	42.37	99.82	11.90	29.13
3	48.09	92.13	12.76	10.80
4	35.85	74.66	10.59	24.62
5	52.21	78.88	Trace	12.80
7	102.29	116.30	21.00	31.92
8	23.06	102.98	10.41	13.84

DISCUSSION

Ratio of lead to arsenic in spray deposits.—Examination of the data presented in the previous paper *(4)* showed that in the 1934 experiments the ratio, Pb: As_2O_3, in the deposits AS APPLIED was 2.1: 1; after the last cover spray application on July 16 the ratio for the trees receiving six cover sprays was 2.59: 1, while on the same date the deposits on the trees receiving but three cover sprays, with the last application on June 16, the ratio was 3.50: 1. At harvest the ratio on the trees receiving six cover sprays was 2.62: 1, and on those receiving three cover sprays 3.05: 1. This appeared to indicate that deposits which were exposed to weathering longest had a higher Pb: As_2O_3 ratio.

In the 1935 experiments, reported in the same paper, the Pb: As_2O_3 ratios were lower, averaging 1.79: 1 as deposited, and 1.85: 1 and 1.81: 1 after the last spray application and at harvest, respectively, showing no significant differences even over a considerable period of time.

Since it has been assumed that acid lead arsenate breaks down to form basic arsenates according to the reaction $5\ PbHAsO_4 + HOH \rightleftharpoons Pb_4\ (PbOH)\ (AsO_4)_3 + 2\ H_3AsO_4$, releasing soluble arsenic, it should follow that the Pb: As_2O_3 ratio should become progressively larger as the decomposition proceeds. A number of workers have considered this point, and the earlier investigations by Hartzell and Wilcoxon *(6)* and Marshall and Ford *(7)* seemed to agree with this theory. More recently, however, Harman *(5)*, Pearce and Avens *(11)*, and Fahey and Rusk *(2)* have found ratios of approximately 2.5: 1 at harvest. Fahey and Rusk were of the opinion that the high ratios reported by some investigators were the result of inaccuracies in sampling and analytical techniques.

In the present work, the Pb: As_2O_3 ratios at harvest were calculated, and are shown in table 8. In addition to the Jonathan apples in 1937 and the Stayman Winesaps in 1938 previously discussed, the analyses of samples taken from a block of Jonathans in 1938 are included. These trees received identical sprays, both as to composition and timing, as the Stayman Winesaps of the same year. To make the comparison more direct, applications to both varieties were made from the same tankful of spray.

The figures given are averages of the analyses of fruits from the tops and bottoms of the trees, a total of 2,800 grams. This amount is sufficiently large (Fahey and Rusk used duplicate samples of 25 fruits) so that the figures should have considerable significance. Explanation of the data, however, is difficult. There appears to be no consistent relationship between the mixture used and the Pb: As_2O_3 ratio, nor does the same variety, Jonathan, show the same ratio in two different years. Spray injury to fruit and to foliage occasioned by these treatments, and reported previously *(12)* bears no apparent relation to the Pb: As_2O_3 ratio as reported in table 8. The most striking comparison, however, is between the two varieties sprayed with identical mixtures in 1938. Here the differences are consistent and relatively large, indicating that weathering may proceed more rapidly on Stayman Winesaps than on Jonathans. A similar circumstance was pointed out in the previous paper between York Imperials and Stayman Winesaps.

Table 8.—Pb: As_2O_3 ratio in residues at harvest.

TREATMENT NUMBERS	1937	1938	
	Jonathan	Stayman	Jonathan
1	3.11	3.48	2.05
2	3.29	2.55	1.71
3	4.23	6.19	2.25
4	2.32	3.22	2.31
5	3.88		2.73
6	2.32		1.98
7	3.32	4.69	2.03
8	5.71	4.84	
MEAN	3.52	4.16	2.15

Timing of spray applications.—Excluding the "dynamite" treatments, the first brood codling moth sprays applied with various stickers and in various schedules during the seasons of 1937 and 1938 exhibited no great differences in either average deposits or retention of lead and arsenic on fruit surfaces. Possible benefits from a "growth schedule" timing were obscured in 1937 by the arrangement that ended both the "growth" and "regular" schedules with a fifth cover spray on the same date. Both schedules maintained high deposits during the critical period of first brood attack.

During 1938, the growth schedule idea was subjected to a more severe test when three applications ending on June 9, treatment 4, were compared with four applications of sprays evenly spaced and ending on June 30, treatment 3. In this case, growth schedule deposits were much reduced by growth of the fruit before the end of the period of codling moth attack.

Spreading and adhesive agents.—In 1937 it was possible directly to compare fish oil, treatment 4, Grasselli Spreader-Sticker, treatment 7, and soybean flour, treatment 8, in their effect both on original deposits and their retention. The charts in figure 2 show that the original deposits of lead and arsenic averaged somewhat higher from the mixture containing fish oil than from the other two. As shown by table 5, the greatest lead residue at harvest appeared in the treatment receiving soybean flour, and the treatment containing fish oil retained the most arsenic. Retention of both poisons was least with Grasselli Spreader-Sticker.

In 1938, a direct comparison was possible between skimmilk powder, treatment 2, and soybean flour, treatment 3. The charts in figure 4 and the data in table 7 indicate that both the deposit and retention from these two mixtures were practically identical.

"Dynamite" sprays.—In 1937, as has already been shown in table 3, the "dynamite" spray, treatment 6, deposited on the average considerably greater quantities of both lead and arsenic in a given spray than did any other. Treatment 5, in which a proprietary compound said to produce the "dynamite type" of deposit was used, deposited less lead than treatment 6, and, averaging the tops and bottoms of the trees, less arsenic. Treatment 5 appeared to produce no greater spray deposits than the average of those from the regular mixtures used in treatments 3, 4, 7, and 8 applied on the same date.

At the time of sampling on July 28, the final sprays in the "dynamite" treatments had been subjected to 32 days of weathering and fruit growth, while only 11 days had elapsed since the final spray application in all other treatments. A comparison of slope of lines in the charts shown in figure 2 indicates that during these respective periods the deposits of lead and of arsenic were lost at a slower rate from the fruits in treatments 5 and 6 than from the others. Because of the longer period of time involved, however, deposits were lowest on July 28 in these "dynamite" treatments. Loss continued to be less rapid, particularly in treatment 6, during the period from July 28 to harvest.

In 1938 the "dynamite" spray idea received its most severe test in treatment 7, table 6, where the second and final cover spray was applied on June 6. The double dosage per tree of an inverted mixture containing 6 pounds of lead arsenate per 100 gallons built up deposits of lead and arsenic greater than those in any of the "non-dynamite" treatments. After 25 days of weathering and of fruit growth, analyses on July 1 showed that these deposits were still greater in the bottoms of the trees and nearly as great in the tops as those produced by two additional "non-dynamite" treatments applied in treatments 1 to 3, and ending on June 30, the day before the analyses were made. Deposits on fruits in the bottoms of the trees in treatment 7 were far higher at this time than in the "non-dynamite" treatments, as may be seen in figure 4. The exact situation in later analyses is somewhat in doubt, but the limited samples analyzed at harvest showed the largest residues in treatment 7, table 7. It might seem that a heavier residue should have been found in treatment 5, which received the standard concentration and dosage of lead arsenate on June 9. In this treatment, however, the double dosage, double concentration, inverted spray was applied on May 31, and the deposit produced had already been reduced one-half or more by growth of fruit before the application of June 6 in treatment 7.

Codling Moth Control.—The average level of spray deposits maintained on the fruits, and the total cover spray gallons applied in the various treatments, are compared with the data relating to codling moth control in tables 9 and 10. For 1938, figures have been taken from a previous publication by Worthley and Steiner *(12)*. The figures for the average level of lead deposits are weighted with respect to time intervals.

In 1937, table 9, the 5-spray schedules exhibited no great differences in codling moth control. The abbreviated "dynamite" schedules employed as much spray material per tree, and gave nearly as good control of codling moth. Fruit growth subsequent to the early date of the last spray application, however, reduced the average deposit of lead per unit of fruit surface during the period considered.

Table 9.—Relation between average level of lead deposit up to July 28, codling moth control, and amounts of spray mixtures used on Jonathans, 1937.

TREATMENT NUMBERS	AVERAGE DEPOSIT	CLEAN FRUIT	WORMS	COVER SPRAY
	Pb, Mg/M² to 7/28	*Per Cent*	*Per 100 Apples*	*Gals. per Tree*
1	243.3	85.6	3.0	60
2	235.3	87.2	1.4	60
3	238.1	90.0	1.3	60
4	246.9	87.7	1.0	60
5	169.9	85.9	3.1	62
6	205.1	88.2	2.7	62
7	220.5	87.6	1.9	60
8	257.4	88.0	1.6	60
Control—no spray		72.7	23.5	
Least significant difference (Odds 20:1)		3.8	2.2	

In 1938, table 10, the abbreviated "dynamite" schedules, treatments 5 and 7, maintained high average deposits with no increase in the amount of spray material used, and without loss of codling moth control. In treatment 6, for which the deposit figures are not available, but which received the first two cover sprays as in treatment 7, only 43 cover spray gallons per tree were used. However, this treatment produced 91 per cent clean fruit and 1.9 worms per 100 apples. A further advantage of the "dynamite" schedule is the saving in labor and machinery costs resulting from the fewer sprays employed. It should be noted that treatment 8 received four late sprays of nicotine bentonite, so that the low deposit of lead is not reflected in an increase in codling moth injury. In an earlier paper, the authors suggested that a level of approximately 125 milligrams of lead per square meter was an index of satisfactory protection against codling moth. While this figure was an approximation, the degree of control being dependent on a number of factors, the figures in table 10 appear to indicate that this level was nearly adequate under the conditions of the 1938 experiments.

Table 10.—Relation between average level of lead deposit up to August 8, codling moth control, and amounts of spray mixtures used on Stayman Winesaps in 1938.

TREATMENT NUMBERS	AVERAGE DEPOSIT	CLEAN FRUIT	WORMS	COVER SPRAY
	Pb, Mg/M² to 8/8	*Per Cent*	*Per 100 Apples*	*Gals. per Tree*
1	130.7	82	2.8	66
2	142.2	89	1.9	70
3	160.5	90	1.4	73
4	147.3	89	3.9	45
5	172.4	90	1.4	66
7	195.7	90	2.5	60
8	94.5	95	1.6	113
Control—no spray		69	33.0	
Least significant difference (Odds 20:1)		5.5	1.2	

Spray Injury.—Data on the extent of injury to the fruits were taken in 1937, and complete data as to the injury to fruits and leaves and the number of dropped fruits in 1938. This information has already been presented by Worthley and Steiner *(12)*. The figures indicated that in both years the injury resulting from the fungicides overshadowed that from the arsenicals. In 1937, those treatments in which "Copper Hydro 40" was used as a fungicide caused relatively severe injury as compared with those containing flotation sulfur; in 1938 the liquid lime sulfur caused considerably greater injury than the copper phosphate mixture containing relatively large amounts of lime. No significant differences in spray injury were apparent between treatments containing the same fungicides.

SUMMARY

The present bulletin reports the results of chemical analyses made to determine the amounts of lead and of arsenic deposited and remaining on the surfaces of apple fruits sprayed with acid lead arsenate in various schedules and mixtures during the seasons of 1937 and 1938. In 1937 the variety used was Jonathan; in 1938 Stayman Winesap. The more interesting points revealed in the study are listed below.

1. Spray deposits of lead and of arsenic were greater on fruits from the bottoms of the trees than on those from the tops. The difference was more marked in 1938 than in 1937 when the operator had been especially trained to spend most of his time in an effort to get thorough spray coverage in the tops of the trees.

2. Calculations of the Pb: As_2O_3 ratio in the data collected gave no consistent figure for either fresh or weathered deposits, nor consistent differences in the ratios exhibited in different spray mixtures. The ratio in treatments to the Jonathan variety was greater in 1937 than in 1938 (3.52 and 2.15). In treatments applied to Stayman Winesaps and to Jonathans from the same tanks of spray mixture in 1938, the ratio was nearly twice as great in the former variety (4.16 and 2.15).

3. In 1937 the spacing of spray applications at progressively longer intervals to compensate for the diminishing growth rate of the fruits, "growth schedule" applications, did not maintain a consistently higher level of spray deposits than the spacing of applications at uniform intervals, when both schedules consisted of five cover sprays ending on the same date.

4. In 1938 the deposits produced by a "growth schedule" of three cover spray applications ending on June 9 were much reduced by subsequent growth of the fruit before the cessation of codling moth attack, as compared with a schedule of four uniformly spaced applications ending on June 30.

5. In 1937 it appeared that the addition of 1 pint of fish oil per 100 gallons of spray mixture resulted in greater deposits of lead and arsenic than the addition of 1 pound of soybean flour. Retention of lead, but not of arsenic, was greater with soybean flour.

6. As compared in 4-cover spray schedules in 1938, soybean flour at 1 pound and skimmilk powder at 0.5 pound per 100 gallons of spray mixture showed little difference in their effect on the deposit and retention of lead and arsenic.

7. In 1937 a schedule of three cover sprays ending on June 26, the last two of which were inverted or "Washington State College dynamite" spray mixture, built up considerably higher deposits of lead and arsenic than any non-inverted spray mixture. In July 28, however, after 32 days of exposure, the deposits were lower than in treatments receiving two additional non-inverted spray applications ending on July 17, and thus analyzed after 11 days of exposure.

8. In 1938 a treatment involving two cover sprays, the second of which was applied on June 6, and comprised a double concentration of lead arsenate in a "modified Washington State College dynamite" spray mixture, applied at a double dosage per tree, showed deposits of lead and arsenic nearly as great as those in treatments receiving the last of four lead arsenate sprays in non-inverted mixtures on June 30, when fruit samples were analyzed on July 1.

9. The "dynamite" treatment mentioned above afforded as good codling moth control as more extended spraying schedules.

BIBLIOGRAPHY

(*1*) Association of Official Agricultural Chemists. 1935. Official and tentative methods of analysis. Ed. 4, 710 pp., illus. Washington, D. C.

(*2*) Fahey, J. E. and H. W. Rusk. 1939. Possible changes in ratio of lead to arsenious oxide in lead arsenate residues on apples. *Jour. Econ. Ent.* **32**: 319-322.

(*3*) Frear, D. E. H. and D. E. Haley. 1934. A simplified method for the rapid determination of lead residues on apples. *Penna. Agr. Exp. Sta. Bull.* **304**.

(*4*) Frear, D. E. H. and H. N. Worthley. 1937. Deposition and retention of sprays on apples. *Penna. Agr. Exp. Sta. Bull.* **344**.

(*5*) Harman, S. W. 1937. Stickers and spreaders used in lead arsenate sprays for codling moth control. *Jour. Econ. Ent.* **30**: 404-407.

(*6*) Hartzell, A. and F. Wilcoxon. 1928. Analyses of sprayed apples for lead and arsenic. *Jour. Econ. Ent.* **21**: 125-130.

(*7*) Marshall, G. E. and O. W. Ford. 1933. The relation of apple spray schedules to the arsenious oxide and lead residues. *Purdue Univ. Agr. Exp. Sta. Bull.* **381**.

(*8*) Marshall, J. 1937. Inverted spray mixtures and their development with reference to codling moth control. *Wash. Agr. Exp. Sta. Bull.* **350**.

(*9*) Marshall, J. and K. Groves. 1938. W. S. C. "Dynamite" spray: How to mix and use it. *Wash. State College Ext. Bull.* **232**.

(*10*) Marshall, J. and K. Groves. 1938. Further experiments with inverted or "dynamite" spray mixtures. *Proc. 33rd Ann. Meeting Wash. State Hort. Assoc.*, pp. 119-126.

(*11*) Pearce, G. W. and A. W. Avens. 1938. The ratio of lead to arsenic in spray residues from lead arsenate. *Jour. Econ. Ent.* **31**: 594-597.

(*12*) Worthley, H. N. and H. M. Steiner. 1939. Apple insect control studies in 1938. *Penna. Agr. Exp. Sta. Bull.* **376**.

BULLETIN 401 OCTOBER 1940

Levels of Protein for Pigs

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

Digest

The most economical protein level for pigs of various weights was studied by feeding them rations containing corn supplemented with low protein tankage, soybean oilmeal, and alfalfa meal. The pigs were fed individually and the feed intake was controlled in order to have a valid comparison between different levels of protein intake.

The results obtained in these experiments are summarized for different weight intervals in order to determine the approximate protein requirements for different stages of growth.

Financially, the most economical protein level for pigs from weaning to the average weight of 70 pounds was found to be 22 per cent. If the price of protein is high and that of corn is low, a ration containing 17 per cent protein may be the most economical. The pigs fed a high protein ration during the period following weaning were more thrifty and stronger than those fed at lower levels.

The most economical level of protein for pigs between the approximate weights of 75 and 125 pounds was found to be between 17 and 20 per cent.

The most economical quantity of protein in the ration for pigs between the weights of 125 and 200 pounds was found to be approximately 15 per cent.

The percentage of protein recommended for pigs above the weight of 100 pounds will depend upon the future use of the pig. If the pig is to be retained for breeding, a high protein ration, regardless of cost, is recommended; but if the pig is to be fattened, the most economical ration is best.

As measured by gain in live weight, the addition of cod liver oil concentrate did not improve a 15 per cent protein ration for growing pigs which had ordinary access to sunlight.

Levels of Protein for Pigs *

T. B. Keith and R. C. Miller†

Rations for pigs consist largely of grains, chief among which is corn. Most of the feed grains which the farmer produces, corn included, are deficient in quantity and quality of protein and must be supplemented with protein from other sources. In many cases supplementary protein meals must be purchased, usually at prices above those of other feeds.

However, it is now possible in most cases to obtain highly efficient protein mixtures by the home mixing of feeds which are either available in the community of the swine producer or obtainable at a reasonable cost.

Soybean oil meal, alfalfa meal, and low protein tankage are such feeds. Because of increased production of soybeans to provide soybean oil for industrial use, an increased quantity of soybean oilmeal is available for livestock feeding. Alfalfa is also more abundant than previously, there having been an increased production of alfalfa of approximately 100 per cent during the last 10 years in Pennsylvania and neighboring states. In the case of feeding tankage, this material was originally produced largely by only the large meat packing companies. In late years, however, small abattoirs have been producing low protein tankage in increasing quantities and now it is readily available in the communities of many feeders.

This bulletin is a report of a series of experiments in which protein was fed at various levels to pigs of different ages and weights, table 1. The sources of protein used to supplement corn were soybean oilmeal, 34 per cent protein tankage, and alfalfa meal.

Chester White, Poland China, Duroc Jersey, and Berkshire pigs were used, the breeds being distributed uniformly in each experiment.

The chemical composition of the yellow corn, soybean oilmeal, and alfalfa was relatively constant. The protein and ash contents of the tankage varied by as much as 3 per cent, table 2.

---

*Authorized for publication September 27, 1940.

†Assistant Professor of Animal Husbandry, and Assistant Professor of Agricultural and Biological Chemistry, respectively.

Table 1.—Outline of experiments for the determination of economical levels of protein for feeding growing pigs.

EXPERIMENT NUMBERS	METHODS OF FEED CONTROL	PROTEIN CONTENTS OF FEED MIXTURES	NUMBER OF INDIVIDUALS	AVERAGE WEIGHT Initial	AVERAGE WEIGHT Final
		per cent		*pounds*	*pounds*
1	pairs	10	10	131	173
		15	10	129	185
2	trios	15	13	82	202
		20	13	85	205
		25	13	86	193
3*	individuals	15	12	53	176
		15	12	48	156
4	trios	12	13	40	186
		17	13	38	220
		22	13	41	204
5	trios	17	10	33	82†
		22	10	32	77†
		27	10	34	83†

*A comparison of a 15 per cent protein ration with the same ration supplemented with cod liver oil.

†These pigs were continued on a 17 per cent protein ration to the average weights of 173, 162, and 165 pounds, respectively.

TANKAGE.—A low protein tankage produced by a local meat packing plant was used in this study. It was made from slaughterhouse waste consisting of material derived from sheep, cattle, and hogs in about equal quantities uniformly throughout the year. This material consisted largely of heads, bones, intestines, and some meat scraps with the addition of a small quantity of pork liver in summer. After being cooked 4 or 5 hours under a steam pressure of 60 pounds, the water was drained off and the tankage cooked another 4 or 5 hours. Then it was dried for a period of approximately five hours and finally ground and sacked.

SOYBEAN OILMEAL.—The soybean oilmeal for these tests was purchased from a large midwestern plant. A greater portion of the soybeans used in its preparation were of the Illini variety grown in central Illinois. They were cleaned and dried to about 8 per cent moisture content before being taken to the plant for processing. Then they were run through rolls and cracked to about one-fourth or one-eighth of their original size and passed through driers to reduce their moisture content to about 2 per cent. The cracked soybeans were next put through Anderson expellers, and the oil was extracted so that the finished meal contained about 5½ per cent of oil. The temperature reached 275° to 280° F. as the beans passed through the expellers to extract the major portion of the oil.

Table 2.—Average chemical composition of feeds used.

FEEDS	DRY MATTER	TOTAL PROTEIN	FAT	CRUDE FIBER	NITROGEN FREE EXTRACT	ASH	CALCIUM	PHOSPHORUS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Yellow corn	88.6	8.9	3.1	1.4	74.0	1.2	.05	.34
Tankage	91.8	33.6	10.0		7.0	41.2	14.86	7.05
Soybean oilmeal	92.1	40.6	5.4	5.7	35.0	5.4	.30	.71
Alfalfa meal	90.2	20.4	1.6	26.4	35.3	6.5	1.48	.30

ALFALFA.—The alfalfa meal used was made from sun dried alfalfa hay grown in central Pennsylvania and contained both the stems and the leaves. Yellow corn of grade No. 2 was used in all experiments.

LOW PROTEIN RATIONS

In experiment 1, a 10 per cent protein ration was compared with a 15 per cent protein ration. The average initial weights of the 2 groups of pigs were 131 and 129 pounds with individual weights ranging from 96 to 157 pounds. The average final weights were 173 and 185 pounds with individual weights ranging from 114 to 208 pounds.

The experiment was conducted between May 5 and June 16, 1936. The tests varied in length from 35 to 42 days because the pigs attained final weights on different dates. Twenty pigs were fed in individual stalls and housed in a colony house, being allowed to run in quarter-acre lots which were free of vegetation. These pigs were fed as 10 matched pairs with respect to sex, breed, and weight, and each member of each pair received the same quantity of feed. One member of each pair was fed the 10 per cent protein ration and the other member was fed the 15 per cent protein ration.

Because of its high fiber content only 4 per cent of alfalfa meal was included in the rations, the quantity being the same in each, table 3.

Table 3.—Feeds and quantity of each used in the 10 and 15 per cent protein rations of experiment 1.

PROTEIN CONTENTS OF RATIONS	CORN	TANKAGE	SOYBEAN OILMEAL	ALFALFA MEAL	SALT*	GROUND LIMESTONE
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
10	93	1	1	4	.5	.5
15	76	15	4	4	.5	.5

*Sodium chloride (common salt)

Data relating to weights, ages, gains and feed consumption are shown in table 4.

Table 4.—Gains, weights, feed consumption, and economy of gains of 10 pairs of pigs; one member of each pair was fed a 10 per cent and the other a 15 per cent protein ration.

PAIR NO.	AGE AT BEGINNING	PROTEIN	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LB. GAIN
	days	*per cent*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	197	10	35	157	205	48	1.37	5.51	193	402
	264	15	35	150	205	55	1.57	5.51	193	351
2	264	10	35	142	191	49	1.40	5.68	199	406
	208	15	35	144	206	62	1.77	5.68	199	320
3	194	10	35	151	198	47	1.34	5.44	190	405
	223	15	35	146	208	62	1.77	5.44	190	307
4	194	10	42	131	181	50	1.19	5.13	216	432
	223	15	42	124	189	65	1.55	5.15	217	334
5	232	10	42	129	188	59	1.40	5.29	222	377
	225	15	42	130	204	74	1.76	5.29	222	300
6	194	10	35	96	114	18	.51	3.16	111	614
	193	15	35	104	142	38	1.09	3.13	109	287
7	193	10	42	123	163	40	.95	4.01	169	421
	232	15	42	107	162	55	1.31	4.00	168	305
8	205	10	42	117	150	33	.78	3.87	163	493
	220	15	42	124	167	43	1.02	3.86	162	377
9	252	10	42	137	184	47	1.12	4.74	199	424
	218	15	42	127	184	57	1.36	4.70	197	346
10	195	10	42	128	160	32	.76	3.79	159	497
	208	15	42	138	185	47	1.12	3.71	156	332
AVERAGE	222	10	39	131	173	42	1.08	4.66	182	447
	193	15	39	129	185	55	1.43	4.65	181	326

The pigs fed the 15 per cent protein ration made, on the average, a 32 per cent greater gain in weight and required 37 per cent less feed for each 100 pounds gain than the pigs fed the 10 per cent protein ration. In every pair the pig fed the 15 per cent protein ration made the greater gain and required less feed per unit of gain. The individual average daily gains of the pigs fed the 15 per cent protein ration ranged from .20 to .58 pound, or from 14 to 114 per cent more than their paired-mates fed the 10 per cent protein ration. The feed required per 100 pounds gain for the individual pigs fed the 15 per cent protein ration ranged from 51 to 327 pounds, or 12 to 53 per cent less than that for the paired mates fed the 10 per cent protein ration, table 4.

HIGH PROTEIN RATIONS

In experiment 2, comparisons were made of the gains in live weight of 3 groups of pigs fed 15, 20, and 25 per cent protein rations, respectively. The average initial weights of the 3 groups were 82, 85, and 86 pounds, with individual weights ranging from 70 to 113 pounds. The average final weights of the 3 groups were 202, 205, and 193 pounds with individual weights ranging from 151 to 231 pounds, table 6.

The experiment was conducted between July 16 and October 8, 1938. Tests of individuals varied in length from 70 to 84 days. Thirty-nine pigs were fed in individual stalls in an experimental barn with concrete floors and concrete outside runways. These pigs were selected in trios of the same breed, sex, and approximate weight. Respective members of each trio were fed 15, 20, and 25 per cent protein rations. The feed allowance was the same for each member of a trio but it varied between different trios. The allowance of feed given to each trio was determined by that member which consumed the least quantity of feed.

The three rations contained the same quantities of alfalfa but the proportions of tankage and soybean oilmeal varied to give the desired quantity of protein in the respective rations, table 5.

Table 5.—Feeds and quantity of each used in the 15, 20, and 25 per cent protein rations of experiment 2.

PROTEIN CONTENTS OF RATIONS	CORN	TANKAGE	SOYBEAN OILMEAL	ALFALFA MEAL	SALT*
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
15	76.5	15	4	4	.5
20	57.5	20	18	4	.5
25	42.5	18	35	4	.5

*Sodium chloride.

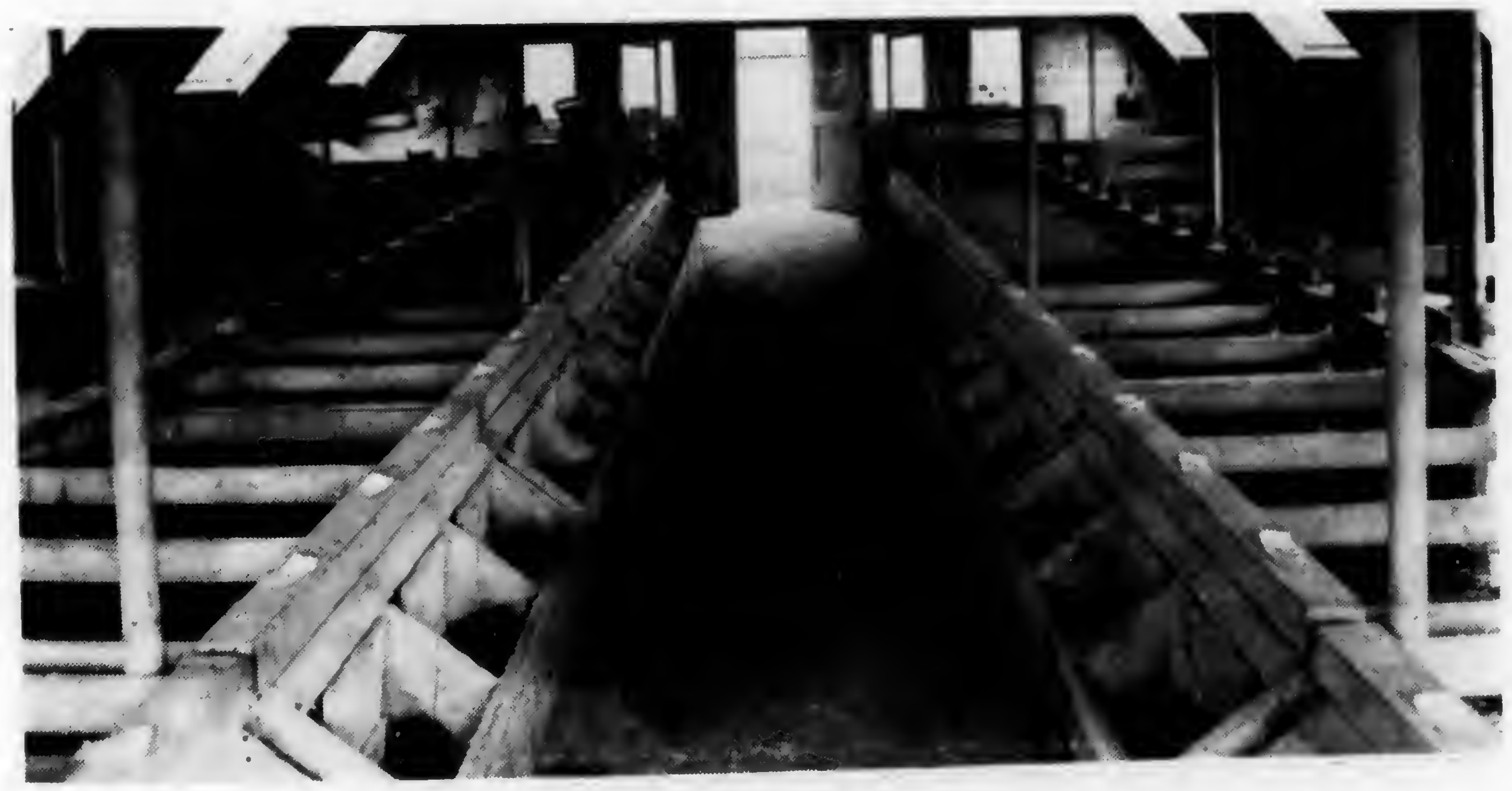


Fig. 1.—The pigs on test were fed in these individual stalls in this experimental barn.

The average daily gains and the average feed required for each 100 pounds of gain were the same, 1.55 pounds and 276 pounds respectively, for the pigs fed the 15 and 20 per cent protein rations. The average daily gains of the pigs fed the 25 per cent protein ration were statistically significantly lower than the average daily gains of the pigs fed the 15 and 20 per cent rations. Of the 13 trios, in 6 trios the member receiving the 15 per cent protein ration, in 6 trios the member receiving the 20 per cent protein ration, and in 1 trio the member receiving the 25 per cent protein ration made the greater gains, table 6.

Table 6.—Gains, weights, feed consumption, and economy of gains of 13 trios of pigs, 1 member of each trio receiving a 15 per cent, 1 a 20 per cent, and 1 a 25 per cent protein ration.

TRIO No.	PROTEIN CONTENTS OF RATIONS	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LBS. GAIN
	per cent	*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	15	156	77	80	192	112	1.45	3.25	251	224
	20	112	77	75	197	122	1.58	3.23	249	204
	25	132	77	76	201	125	1.62	3.25	250	200
2	15	132	70	83	198	115	1.64	4.26	299	260
	20	132	70	88	209	121	1.73	4.27	299	247
	25	132	70	84	175	91	1.30	4.27	299	328
3	15	132	70	94	207	113	1.61	4.35	305	270
	20	130	70	95	217	122	1.74	4.36	306	250
	25	130	70	92	192	100	1.43	4.38	307	307
4	15	130	84	73	210	137	1.63	4.57	384	280
	20	105	84	76	204	128	1.52	4.58	385	301
	25	108	84	74	203	129	1.54	4.58	385	298
5	15	130	77	79	208	129	1.67	4.25	327	254
	20	130	77	84	210	126	1.64	4.24	326	259
	25	130	77	88	196	108	1.40	4.24	326	302
6	15	116	84	75	213	138	1.64	4.80	370	268
	20	105	84	83	189	106	1.26	4.81	370	349
	25	105	84	76	200	124	1.48	4.80	370	298
7	15	101	77	80	213	133	1.73	4.50	347	261
	20	130	77	81	208	127	1.65	4.50	347	273
	25	130	77	94	212	118	1.53	4.51	347	294
8	15	128	70	95	200	105	1.50	4.43	310	295
	20	130	70	113	231	118	1.69	4.43	310	263
	25	130	70	112	209	97	1.39	4.45	312	321
9	15	100	84	70	190	120	1.43	4.34	364	304
	20	101	84	71	197	126	1.50	4.35	365	290
	25	128	84	74	198	124	1.48	4.34	365	294
10	15	128	84	74	206	132	1.57	4.47	375	284
	20	100	84	72	199	127	1.51	4.41	371	292
	25	100	84	73	179	106	1.26	4.45	374	353
11	15	137	70	98	202	104	1.49	4.28	299	288
	20	137	70	110	215	105	1.50	4.28	299	285
	25	137	70	102	186	84	1.20	4.28	299	356
12	15	137	84	82	216	134	1.59	4.09	344	256
	20	122	84	71	179	108	1.29	4.08	342	317
	25	122	84	73	151	78	.93	4.10	345	442
13	15	122	77	82	176	94	1.22	4.10	316	336
	20	137	77	91	209	118	1.53	4.13	318	269
	25	137	77	95	206	111	1.44	4.09	316	284
AVERAGE	15	126	77	82	202	120	1.55	4.28	330	276
	20	121	77	85	205	120	1.55	4.28	330	276
	25	125	77	86	193	108	1.38	4.29	330	314

The most economical ration for the pigs of this experiment for weights between approximately 82 and 128 pounds was studied, table 7. The data relating to gains and to feed consumption of the pigs were obtained by using the weights of each individual from the beginning of the experiment to the weekly weight at which the pig first attained 125 pounds.

Table 7.—Weights, gains, and economy of gains of pigs between the average initial weight of 82 and the average final weight of 128 pounds, fed the 15, 20, and 25 per cent protein rations.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED FOR EACH 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
13	15	35	82	128	1.34	3.53	269
13	20	31	85	128	1.39	3.42	252
13	25	33	86	127	1.28	3.58	284

The pigs fed the 20 per cent protein ration made more rapid gains on less feed and in less time than the pigs fed the 15 or the 25 per cent protein ration. The pigs fed the 20 per cent protein ration made .05 pound greater average daily gain, 3 per cent more in 4 days or similar gains in 11 per cent less time, than the pigs fed the 15 per cent protein ration. They ate an average of .11 pound or 3 per cent less feed per day, and required an average of 17 pounds or 6 per cent less feed for each 100 pounds of gain than the pigs fed the 15 per cent protein ration.

The optimum percentage of protein required in a ration for pigs from 125 pounds to approximately 200 pounds was studied, table 8. The data relating to gains and to feed consumption of the pigs of these weights were obtained by using values for each individual from the weekly weight at which the pig first surpassed 125 pounds to the weekly weight at which the pig first reached about 200 pounds.

The pigs fed the 15 per cent protein ration made about the same average daily gain on approximately the same quantity of feed for each 100 pounds gain in 28 per cent less time than the pigs fed the

Table 8.—Weights, gains, and economy of gains of pigs between the average initial weight of 127 and the average final weight of 207 pounds that were fed 15, 20, and 25 per cent protein rations.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED FOR EACH 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
7	15	39	128	196	1.76	4.71	268
7	20	46	128	207	1.73	4.58	265
7	25	45	127	193	1.49	4.60	316

20 per cent protein ration. The pigs fed the 25 per cent protein ration made the slowest average daily gain and required the greater quantity of feed for each 100 pounds gain.

MEDIUM PROTEIN WITH COD LIVER OIL

In experiment 3, a comparison was made in gain in live weight of pigs fed the 15 per cent protein ration used in experiments 1 and 2 with and without cod liver oil. Twenty-four pigs were divided equally as to breed, sex, and approximate weight into 2 groups of 12 each. All pigs were full fed in individual stalls in the experimental barn. One group received 1 per cent of cod liver oil concentrate* in its ration, and all pigs had ordinary access to sunlight. The average initial weights of the 2 groups were 53 and 48 pounds with individual weights ranging from 31 to 75 pounds. The average final weights of the two groups were 176 and 156 pounds with individual weights ranging from 86 to 225 pounds, table 9.

---

*A blend of natural vitamin A and D concentrate and non-destearinated cod liver oil. Guaranteed 3000 U. S. P. units vitamin A and 400 U. S. P. units vitamin D per gram

Table 9.—Gains, weights, feed consumption, and economy of gains of 2 groups of pigs full fed individually; 1 group received a 15 per cent protein ration without cod liver oil, and 1 group received a 15 per cent protein ration with cod liver oil.

PIG NO.	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LB. GAIN
No Cod Liver Oil									
	days	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	122	108	56	206	150	1.39	2.71	293	195
2	135	98	71	218	147	1.50	2.83	278	189
3	123	108	49	156	107	.99	2.71	293	274
4	123	108	48	121	73	.67	2.77	299	410
5	123	108	50	173	123	1.14	2.77	299	243
6	122	108	52	167	115	1.06	2.78	301	262
7	123	108	43	170	127	1.16	2.62	283	223
8	155	108	56	193	137	1.27	2.76	299	218
9	116	108	49	180	131	1.21	2.55	276	211
10	117	108	36	124	88	.81	2.70	292	332
11	147	108	69	184	115	1.06	2.73	295	256
12	120	108	56	219	163	1.50	3.03	328	201
AVERAGE	127	107	53	176	123	1.15	2.75	295	251
Cod Liver Oil									
1	123	108	37	160	123	1.14	2.70	292	237
2	123	108	54	169	115	1.06	2.78	301	261
3	139	108	44	136	92	.85	2.48	269	292
4	123	42	46	86	40	.97	3.55	149	372
5	135	108	75	225	150	1.39	2.78	302	201
6	123	108	58	125	67	.62	2.53	274	409
7	123	108	56	193	137	1.27	2.56	277	202
8	118	108	53	205	152	1.40	2.77	300	197
9	116	108	44	151	107	1.00	2.52	275	255
10	155	108	31	135	104	.96	2.64	285	274
11	155	108	35	104	69	.64	2.72	294	426
12	120	108	46	180	134	1.24	2.95	319	238
AVERAGE	129	102	48	156	108	1.04	2.74	278	280

This experiment was conducted between January 27 and May 15, 1938. The tests of individual pigs varied from 42 to 108 days.

The pigs fed the 15 per cent protein ration without the cod liver oil concentrate made .11 pound or 10 per cent more average daily gain and required 29 pounds or 10 per cent less feed for each 100 pounds gain than pigs fed the 15 per cent protein ration with cod liver oil. This difference is not great enough to be considered significant.

MEDIUM PROTEIN RATIONS

In experiment 4, a comparison was made in gains in live weight of 3 groups of pigs fed 12, 17, and 22 per cent protein rations respectively. The average initial weights of the 3 groups were 40, 38, and 41 pounds, with individual weights ranging from 24 to 58 pounds. The average final weights of the 3 groups were 186, 220, and 204 pounds with individual weights ranging from 136 to 245 pounds.

The experiment was conducted from May 24 to November 7, 1938, a period of 167 days, under the same conditions and the same method of feed control as was used in experiment 2. In this experiment the pigs were fed 3 times instead of 2 times daily as in experiments 1 and 2.

The same ingredients as in experiments 1 and 2 were used. Four per cent of alfalfa was included in each ration, table 10.

Table 10.—Feeds and quantity of each used in the 12, 17, and 22 per cent protein rations of experiment 4.

PROTEIN CONTENTS OF RATIONS	CORN	TANKAGE	SOYBEAN OILMEAL	ALFALFA MEAL	SALT*
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
12	87.5	4	4	4	.5
17	69.5	13	13	4	.5
22	51.5	22	22	4	.5

*Sodium chloride.

The pigs fed the 17 per cent protein ration made the most rapid gains and required the smallest quantity of feed for each 100 pounds of gain, even though the feed requirements of the pigs fed the 12 per cent and the 22 per cent protein rations were also quite low. The average daily feed intake in this experiment was considerably lower than the average daily intake in experiments 1 and 2. In the latter experiments, the average daily feed intake was 4.66 and 4.28 pounds respectively, as compared to 2.76 pounds for experiment 4. Evidently two factors were responsible for some of this difference: smaller initial weights of the pigs, and poor appetites of the members of the trios fed the 12 per cent protein ration, table 11.

Table 11.—Gains, weights, feed consumption, and economy of gains of 13 trios of pigs, 1 member of each trio receiving a 12 per cent, 1 a 17 per cent, and 1 a 22 per cent protein ration.

TRIO NO.	PROTEIN CONTENTS OF RATIONS	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LB. GAIN
	per cent	*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	12	81	167	35	156	121	.73	2.58	430	355
	17	64	167	38	217	179	1.07	2.58	430	240
	22	64	167	42	222	180	1.08	2.57	429	238
2	12	81	167	41	237	196	1.17	2.72	453	231
	17									
	22	81	167	43	215	172	1.03	2.77	460	267
3	12									
	17	58	167	24	198	174	1.04	2.76	459	264
	22	58	167	25	192	167	1.00	2.75	458	275
4	12	58	167	30	136	106	.63	2.59	432	408
	17	58	167	33	207	174	1.04	2.67	445	256
	22	64	167	32	212	180	1.08	2.67	446	248
5	12	81	167	36	218	182	1.09	3.01	501	275
	17	64	167	37	219	182	1.09	3.01	503	276
	22	64	167	39	225	186	1.11	3.03	505	271
6	12									
	17	64	167	40	210	170	1.02	2.62	437	257
	22									
7	12									
	17	72	167	33	219	186	1.11	2.73	455	245
	22	88	167	41	195	154	.92	2.73	457	297
8	12	88	167	42	207	165	.99	2.85	476	288
	17	57	167	43	207	164	.98	2.86	476	290
	22	57	167	43	201	158	.95	2.87	479	303
9	12	60	167	31	127	96	.57	2.75	458	477
	17	72	167	37	217	180	1.08	2.76	459	255
	22									
10	12	85	162	49	221	172	1.06	2.92	474	276
	17	85	162	48	241	193	1.19	2.91	471	244
	22	85	162	54	211	157	.97	2.84	461	294
11	12									
	17	81	162	45	238	193	1.19	2.47	400	207
	22	81	162	45	198	153	.94	2.48	402	263
12	12	81	167	34	178	144	.86	2.83	472	328
	17	81	167	34	221	187	1.12	2.84	473	253
	22	81	167	39	209	170	1.02	2.82	468	275
13	12	81	167	58	194	136	.81	2.83	473	348
	17	81	167	46	245	199	1.19	2.86	477	240
	22	81	167	43	163	120	.72	2.69	449	374
AVERAGE	12	77	166	40	186	146	.88	2.79	463	332
	17	70	166	38	220	182	1.09	2.76	457	252
	22	67	166	41	204	163	.98	2.75	440	282

Among the 13 pigs fed the 12 per cent protein ration, only 7 reached a final weight of 150 pounds, or above, within 166 days. One pig fed the 17 per cent protein ration and 2 pigs fed the 22 per cent protein ration failed to reach 150 pounds within 166 days.

The protein requirements of pigs having an initial weight of 38 pounds and being carried to a weight of 75 pounds were studied, table 12. The data relating to gains and to feed consumption of the pigs were obtained by using the weights of each individual from its initial weight to the weekly weight at which the pig first attained 70 pounds.

Table 12.—Weights, gains, and economy of gains of the pigs between the average initial weight of 38 and the average final weight of 75 pounds, being fed 12, 17, and 22 per cent protein rations.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED FOR EACH 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	12	76	40	74	.45	1.56	375
12	17	72	38	75	.50	1.43	301
12	22	70	41	74	.51	1.31	289

The pigs fed the 22 per cent protein ration made .06 pound greater average daily gain, 13 per cent more in 6 days or similar gains in 8 per cent less time, than the pigs fed the 12 per cent protein ration. They ate an average daily ration of .25 pound or 16 per cent less, and they required 86 pounds or 23 per cent less feed for each 100 pounds of gain, than the pigs fed the 12 per cent protein ration. The pigs fed the 17 per cent protein ration made .05 pound greater average daily gain, 11 per cent more in 4 days or similar gains in 5 per cent less time, than the pigs fed the 12 per cent protein ration.

They ate an average daily ration of .13 pound or 8 per cent less and required 74 pounds or 19 per cent less feed for each 100 pounds of gain than the pigs fed the 12 per cent ration. The pigs fed the 22 per cent protein ration made .01 pound greater average daily gain, 2 per cent more gain in 2 days or similar gains in 3 per cent less time, than the pigs fed the 17 per cent protein ration. They ate an average daily ration of .12 pound or 8 per cent less and required 12 pounds or 4 per cent less feed for each 100 pounds gain, than the pigs fed the 17 per cent protein ration.

Results obtained with pigs weighing 73 pounds and being carried to a weight of 131 pounds are given in table 13. The data relating to gain and to feed consumption of the pigs of these weights were obtained by using values for each individual for the weekly weight at which the pig first attained 70 pounds and the weekly weight at which the pig first surpassed 125 pounds.

Table 13.—Weights, gains, and economy of gains of the pigs, between the average initial weight of 73 pounds and the average final weight of 131 pounds, being fed 12, 17, and 22 per cent protein rations.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED FOR EACH 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	12	54	73	128	1.02	3.20	310
12	17	45	75	131	1.27	3.08	245
11	22	48	75	128	1.13	2.86	259

The pigs fed the 17 per cent protein ration made .25 pound or .21 per cent greater average daily gain in 9 days or similar gains in 17 per cent less time than the pigs fed the 12 per cent protein ration. They ate an average daily ration of .12 pound or 4 per cent less total feed and required 65 pounds or 21 per cent less for each 100 pounds gain than the pigs fed the 12 per cent protein ration. The pigs fed the 17 per cent protein ration made more economical gains than the pigs fed the 22 per cent protein ration, and the pigs fed the 22 per cent protein ration made more economical gains than the pigs fed the 12 per cent protein ration.

Results are summarized in table 14 for pigs from a weight of 130 pounds to a weight of 220 pounds. The data relating to gains and to feed consumption of the pigs were obtained by using the weights of each individual for the weekly weight at which the pig first attained 125 pounds and the average final weekly weight of approximately 215, 220, and 208 pounds for the respective groups.

Table 14.—Weights, gains, and economy of gains of pigs between the average initial weight of 128 pounds and the average final weight of 220 pounds, fed 12, 17, and 22 per cent protein rations.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED FOR EACH 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
5	12	53	130	215	1.59	4.63	282
12	17	49	131	220	1.84	4.52	245
10	22	55	128	208	1.56	4.47	302

The pigs fed the 17 per cent protein ration made the most economical and the most rapid gains. These pigs made a .25 pound or 16 per cent greater average daily gain in 4 days, or similar gains in 8 per cent less time, with an average daily ration of .11 pound or 2 per cent less than the pigs fed the 12 per cent protein ration. They required 37 pounds or 13 per cent less feed for each 100 pounds of gain than the pigs fed the 12 per cent protein ration. The pigs fed the 22 per cent protein ration made more economical gains than those fed the 12 per cent protein ration but more expensive gains than the pigs fed the 17 per cent protein ration.

HIGH PROTEIN FROM WEANING

In experiment 5, a comparison was made in gains in live weight of 3 groups of pigs fed 17, 22, and 27 per cent protein rations during the period from weaning to 80 pounds live weight. Following this, a comparison was made of the gains in live weight of these same pigs from 80 pounds to a final weight of approximately 170 pounds during which time all of the pigs were fed a 17 per cent protein ration. The average initial weights of the 3 groups were 33, 32, and 34 pounds, with individual weights ranging from 27 to 40 pounds. The average final weights of the 3 groups were 82, 77, and 83 pounds with individual weights ranging from 54 to 98 pounds. Each pig was fed for a period of 63 days. At the end of 63 days all members of each trio were fed a 17 per cent protein ration until the end of the experiment. This experiment was conducted between May 24 and October 4, 1939, a total period of 133 days.

The feeds used in these rations were the same as those in the previous experiments. The quantity of alfalfa was increased from 4 to 10 per cent, table 15.

Table 15.—Feeds and quantity of each used in the 17, 22, and 27 per cent protein rations in experiment 5.

PROTEIN CONTENTS OF RATIONS	CORN	TANKAGE	SOYBEAN OILMEAL	ALFALFA MEAL	SALT*
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
17	65.5	12	12	10	.5
22	45.5	22	22	10	.5
27	36.5	20	33	10	.5

*Sodium chloride.

The pigs fed the 17 per cent protein ration showed a higher rate of gain than that of the pigs fed the 22 per cent protein ration and a rate of gain equal to that of the pigs fed the 27 per cent protein ration. The rate of gain of each of the three groups is considered to be normal. The feed requirements for 100 pounds of gain for the 3 groups were lower than are usually reported for average feed requirements of pigs of this age and weight. The average daily gains and the feed required for each 100 pounds of gain of the 3 groups fed the 17, 22, and 27 per cent protein rations were not significantly different, table 16.

A further study was made of the economy of gains of the three groups of pigs from the average weights of approximately 82, 77, and 83 pounds to average weights of 173, 163, and 165 pounds respectively, each group being fed the 17 per cent protein ration, table 17.

The pigs fed the 17 per cent protein ration during the period previous to attaining a weight of 82 pounds made more economical gains than the pigs fed the 22 and 27 per cent protein rations.

Table 16.—Gains, weights, feed consumption, and economy of gains of 10 trios of pigs, 1 member of each trio receiving a 17 per cent, 1 member a 22 per cent, and 1 a 27 per cent protein ration, for a period of 63 days.

TRIO No.	PROTEIN CONTENTS OF RATIONS	AGE	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	17	72	38	81	43	.68	2.11	94.1	309
	22	84	36	87	51	.81	2.11	93.1	260
	27	72	38	87	49	.78	2.11	93.1	271
2	17	84	37	98	61	.97	2.63	73.9	272
	22	84	36	96	60	.95	2.62	69.8	276
	27	72	36	87	51	.81	2.60	72.8	321
3	17	84	30	85	55	.87	2.36	92.4	270
	22	84	34	91	57	.90	2.24	81.4	248
	27	84	32	83	51	.91	2.32	99.5	286
4	17	72	24	55	31	49	1.51	95.1	307
	22	72	21	54	33	.52	1.54	95.1	288
	27	72	28	66	38	.60	1.51	96.1	253
5	17	84	35	91	56	.89	2.25	101.0	180
	22	84	33	82	49	.78	2.23	100.6	287
	27	72	32	82	50	.79	2.25	100.6	283
6	17	73	30	72	42	.67	1.84	94.9	276
	22	73	29	66	37	.59	1.61	80.9	275
	27	73	30	83	53	.84	2.18	116.7	259
7	17	76	37	88	51	.81	2.06	79.4	255
	22	76	37	61	24	.38	2.06	84.9	542
	27	76	40	89	49	.78	2.06	79.6	265
8	17	76	31	82	51	.81	2.01	10.6	248
	22	76	32	82	50	.79	2.06	10.9	260
	27	76	34	76	42	.67	2.04	10.8	307
9	17	73	31	78	47	.75	2.07	89.4	278
	22	73	32	77	45	.71	2.07	89.4	290
	27	73	34	88	54	.86	2.01	85.9	235
10	17	84	36	88	52	.82	2.19	80.2	265
	22	84	27	73	46	.73	2.06	71.7	291
	27	84	38	92	54	.86	2.18	76.8	254
AVERAGE	17	78	33	82	50	.78	2.06	90.6	266
	22	78	32	77	45	.72	2.06	87.6	302
	27	75	34	83	49	.78	2.13	92.9	273

The data shown in table 18 indicate that the 17 per cent protein ration is more economical for pigs between the weights of 32 and 82 pounds than the 22 or 27 per cent protein rations, since the pigs fed the 17 per cent protein ration made more rapid and economical gains over the entire period of growth up to 173 pounds. The favorable influence of the 17 per cent protein ration was therefore reflected in the period of growth ensuing after the weight of approximately 82 pounds was attained.

Table 17.—Average weights, gains, feed consumption, and economy of gains of 3 groups of pigs, between the average initial weight of 77 and the average final weight of 173 pounds, all 3 groups receiving the 17 per cent protein ration for a period of 70 days.

NUMBER OF PIGS	LEVEL OF PROTEIN INTAKE IN PREVIOUS PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	FEED PER 100 LB. GAIN
	per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
10	17	82	173	1.30	4.46	345
8	22	77	163	1.25	4.52	366
10	27	83	165	1.18	4.47	380

Table 18.—Weights, gains, feed consumption, and feed requirements of pigs fed 17, 22, and 27 per cent protein rations from weights at weaning to 70 pounds; then a 17 per cent protein ration, to all groups, for the remainder of the experiment for a total period of 133 days.

NUMBER OF PIGS	PROTEIN CONTENTS OF RATIONS	AGE	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	AVERAGE DAILY GAIN	AVERAGE DAILY RATION	TOTAL FEED	FEED PER 100 LB. GAIN
	per cent	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
10	17	78	33	173	140	1.05	3.34	445	318
9	22	78	32	163	130	.92	3.34	445	342
10	27	75	34	165	131	.98	3.36	447	342

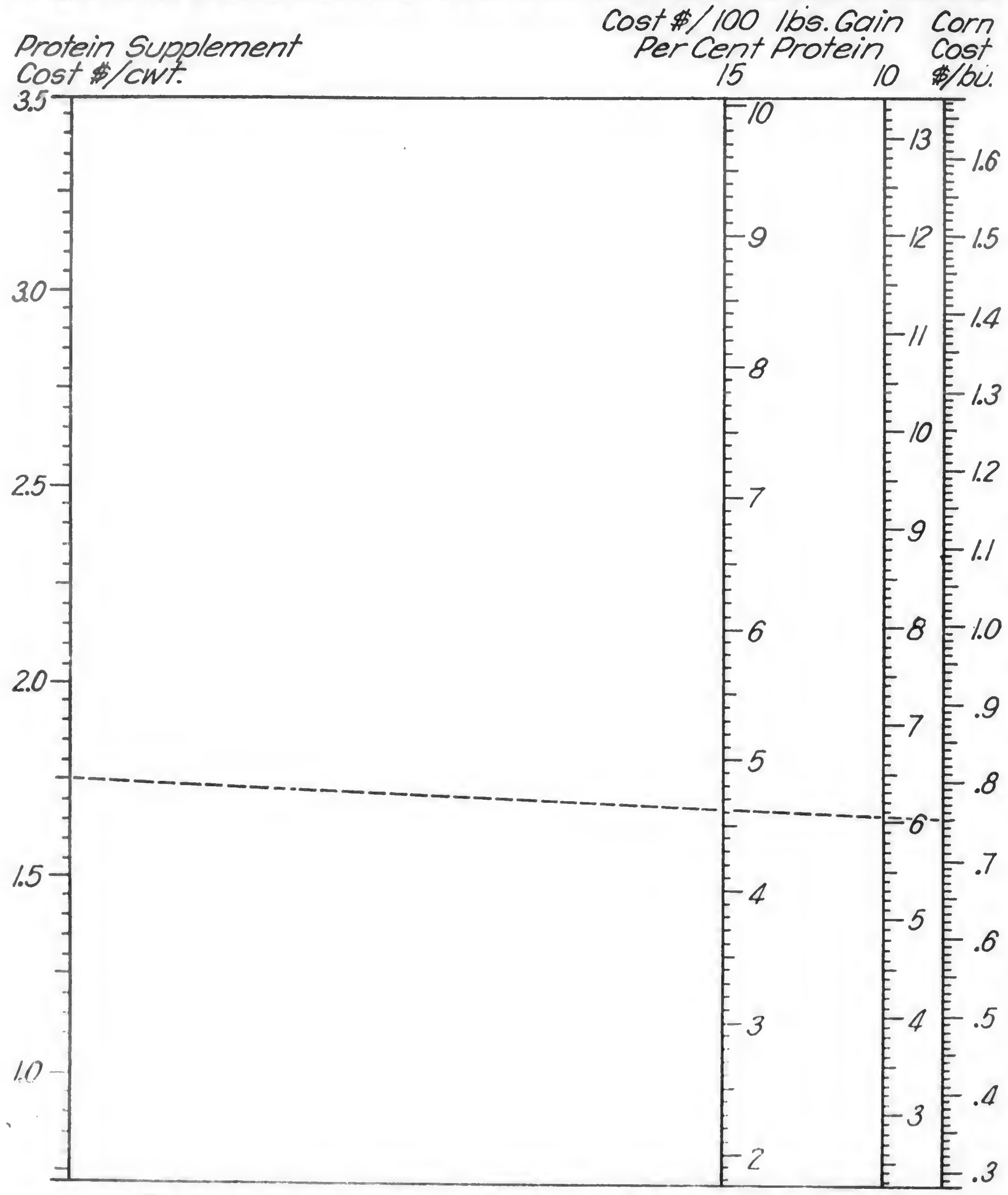
Nomographs are presented which are based on the results obtained in these experiments. These charts are useful in determining costs of gains at various protein levels under a wide range of feed prices.

Nomograph 1 presents the comparative cost of 100 pounds of gain of 2 groups of pigs between the approximate weights of 130 to 185 pounds, when fed 15 and when fed 10 per cent protein rations, table 4. For example, if it is desired to know the most economical level of protein to feed with a supplement mixture costing $1.75 per 100 pounds and corn at 75 cents per bushel, a straight edge is placed across the chart at $1.75 at the left-hand side and 75 cents at the right-hand side. The points of intersection on the 15 per cent protein scale and the 10 per cent protein scale indicate the costs of 100 pounds of gain, which are $4.62 and $6.05, respectively.

Nomographs 2 and 3 cover the costs of gains for pigs from an initial weight of approximately 80 pounds to a final weight of approximately 128 pounds, and from an initial weight of 128 pounds to a final weight of about 200 pounds, for rations containing 25, 20, and 15 per cent protein. The data used in making nomographs 2 and 3 were taken from tables 7 and 8 respectively.

Nomograph 4 presents comparative costs of 100 pounds of gain for pigs weighing approximately 38 to 74 pounds, for which data are given in table 12. These pigs were fed 22, 17, and 12 per cent protein rations. For example, if it is desired to determine the most economical level of protein to feed with a supplement mixture at $1.75 per hundred pounds and corn at 75 cents per bushel, a straight edge is placed across the chart at $1.75 at the left-hand side and 75 cents at the right-hand side. The points of intersection on the 22 per cent protein scale, the 17 per cent protein scale, and

Nomograph 1: For the rapid calculation of the feed cost of 100 pounds gain of pigs from an average initial weight of 130 pounds to an average final weight of 185 pounds when fed 10 and when fed 15 per cent protein rations.*

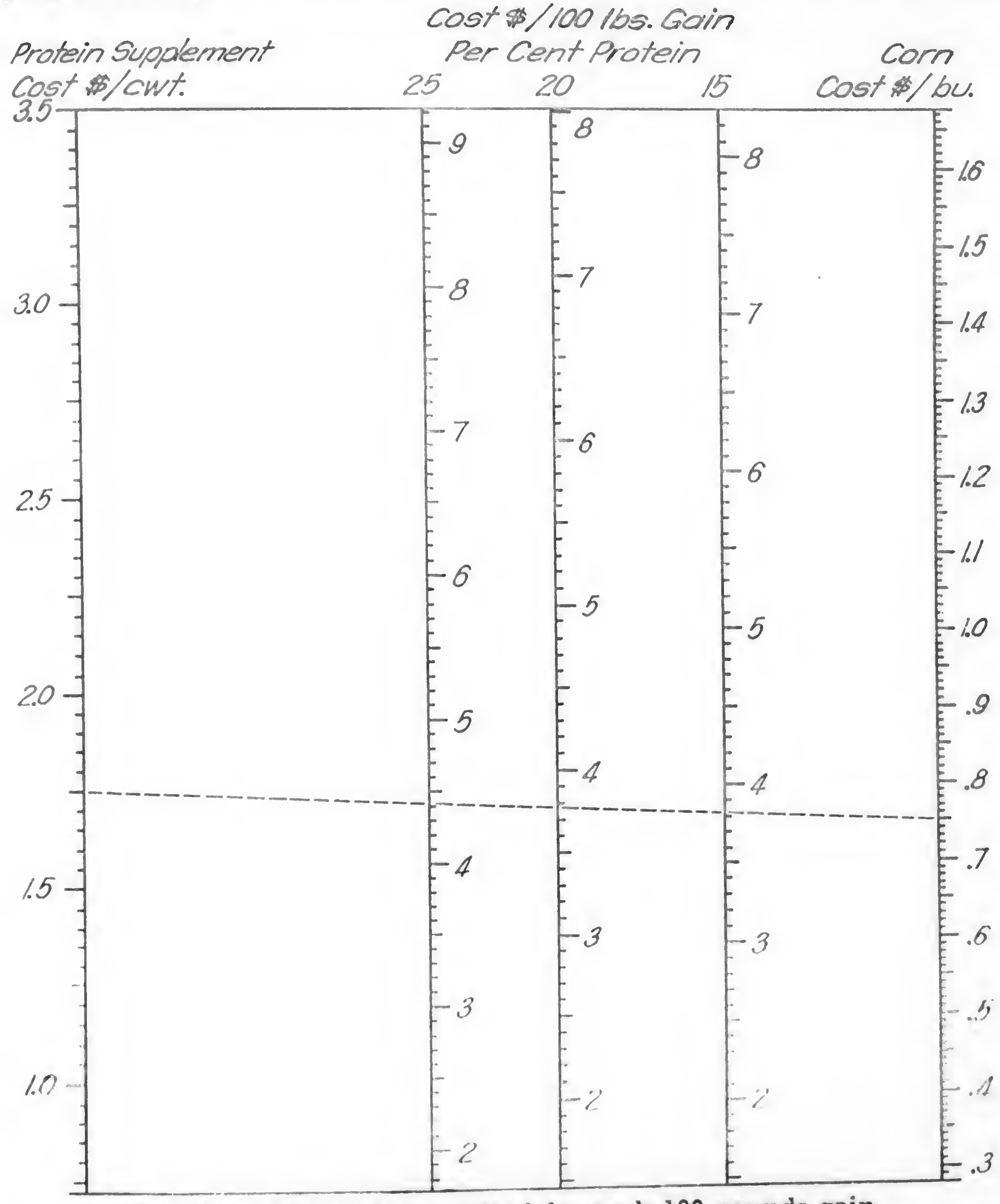


*Feeds and quantity required for each 100 pounds gain.

Per Cent Protein in Ration	15	10
Feeds	*pounds*	*pounds*
Corn	247.9	415.7
Tankage	48.9	4.5
Soybean Oilmeal	13.0	4.5
Alfalfa meal	13.0	17.9
Salt	1.6	2.2
Ground limestone	1.6	2.2
Total	326.0	447.0

*Note that the ration having the highest level of protein is the left hand (first) scale in all of the nomographs.

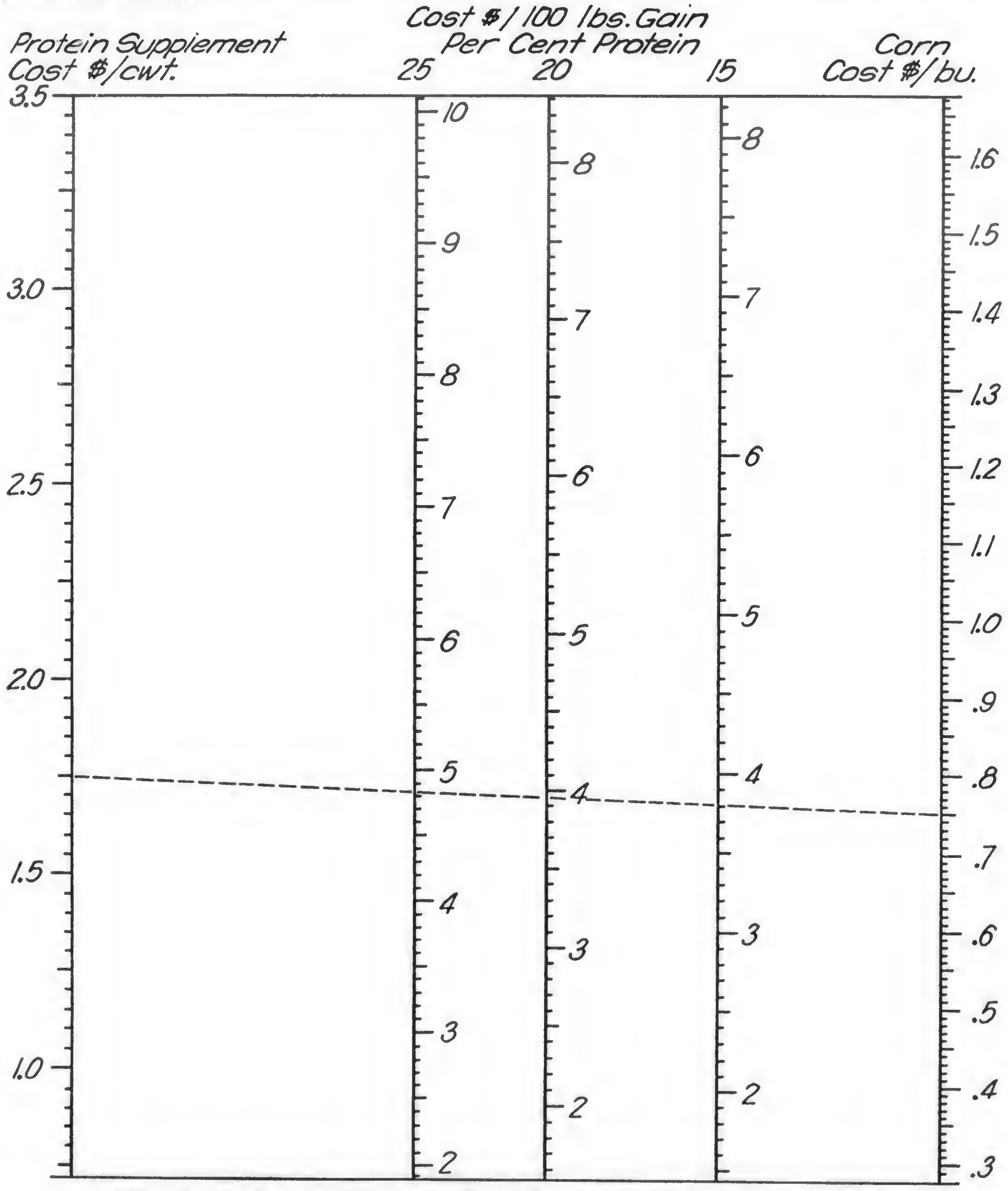
Nomograph 2: For the rapid calculation of the feed cost of 100 pounds gain of pigs from an average initial weight of 82 pounds to an average final weight of 128 pounds when fed 15, when fed 20, and when fed 25 per cent protein rations.*



*Feeds and quantity required for each 100 pounds gain.

Per Cent Protein in Ration	25	20	15
Feeds	*pounds*	*pounds*	*pounds*
Corn	120.7	144.9	205.7
Tankage	51.1	50.4	40.4
Soybean oilmeal	99.4	45.4	10.8
Alfalfa meal	11.4	10.1	10.8
Salt	1.4	1.2	1.3
Total	284.0	252.0	269.0

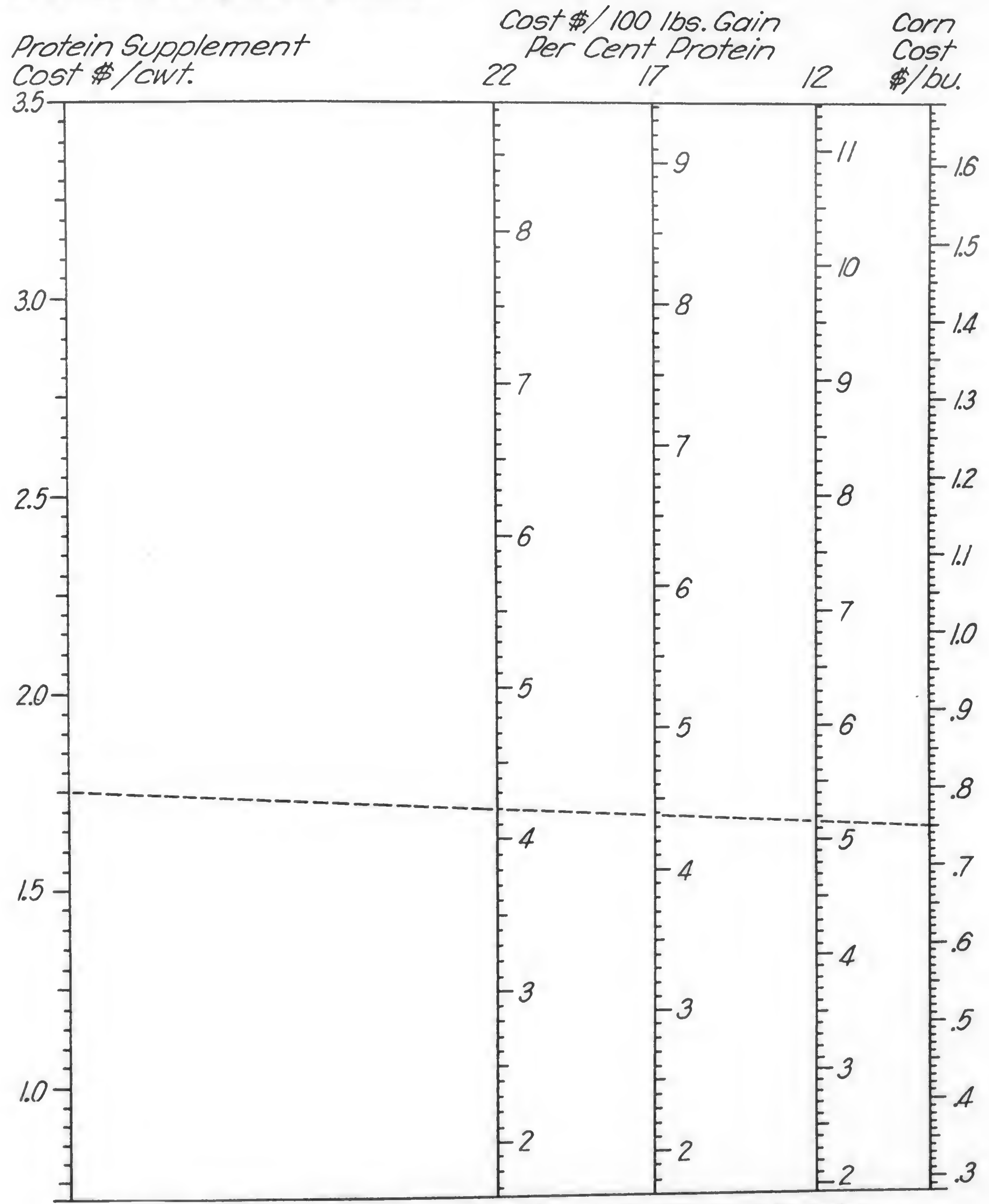
Nomograph 3: For the rapid calculation of the feed cost of 100 pounds gain of pigs from an average initial weight of 128 pounds to an average final weight of 200 pounds when fed 15, when fed 20, and when fed 25 per cent protein rations.*



***Feeds and quantity required for each 100 pounds gain.**

PER CENT PROTEIN IN RATION	25	20	15
Feeds	*pounds*	*pounds*	*pounds*
Corn	134.3	152.4	205.0
Tankage	56.9	53.0	40.3
Soybean oilmeal	10.6	47.7	10.7
Alfalfa meal	12.6	10.6	10.7
Salt	1.6	1.3	1.3
TOTAL	316.0	265.0	268.0

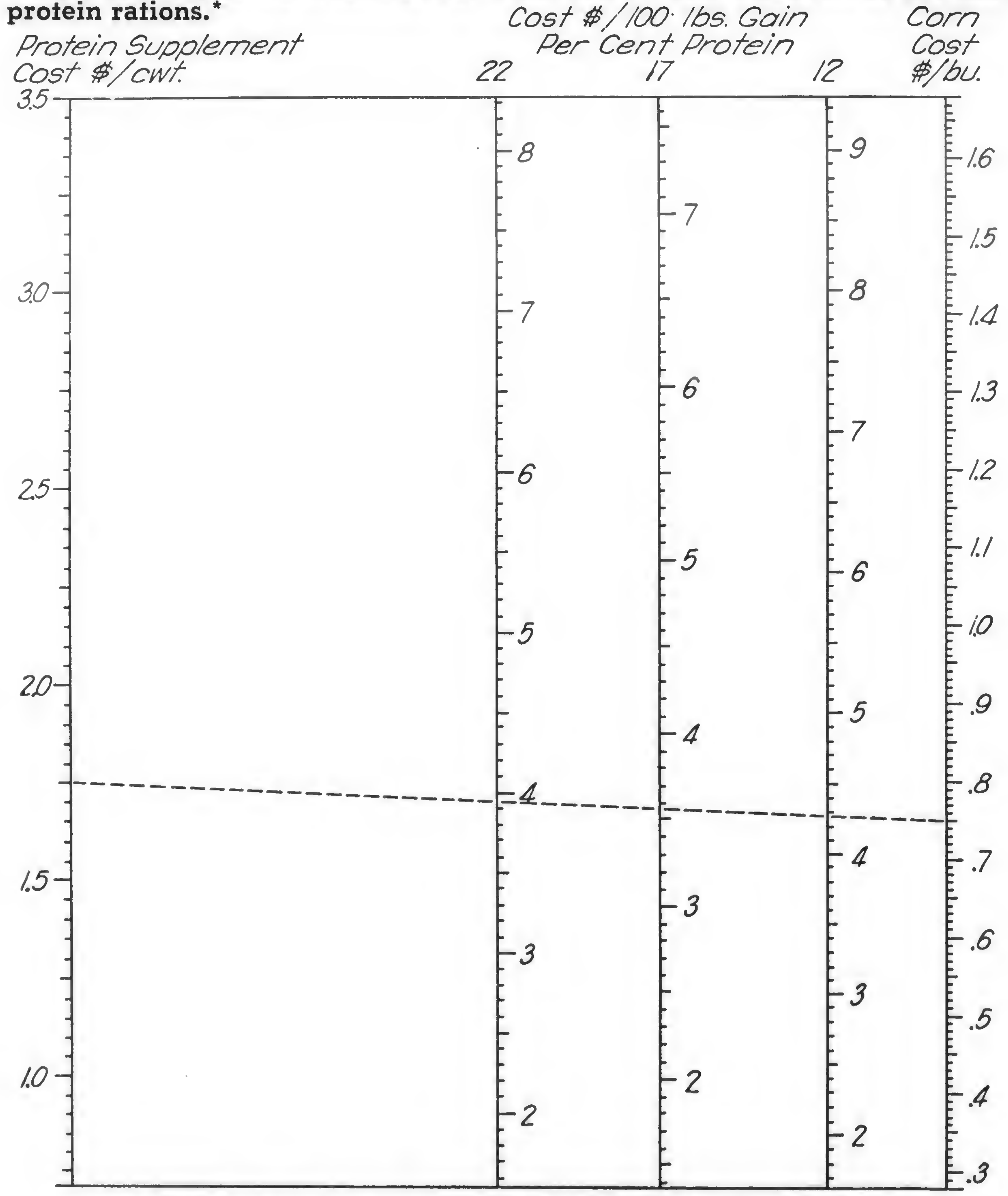
Nomograph 4: For the rapid calculation of the feed cost of 100 pounds of gain of growing pigs from an average initial weight of 38 pounds to an average final weight of 75 pounds, when fed 12, when fed 17, and when fed 22 per cent protein rations.*



***Feeds and quantity required for each 100 pounds gain.**

PER CENT PROTEIN IN RATION	22	17	12
Feeds	*pounds*	*pounds*	*pounds*
Corn	148.8	209.3	328.1
Tankage	63.6	39.1	15.0
Soybean oilmeal	63.6	39.1	15.0
Alfalfa meal	11.6	12.0	15.0
Salt	1.4	1.5	1.9
TOTAL	289.0	301.0	375.0

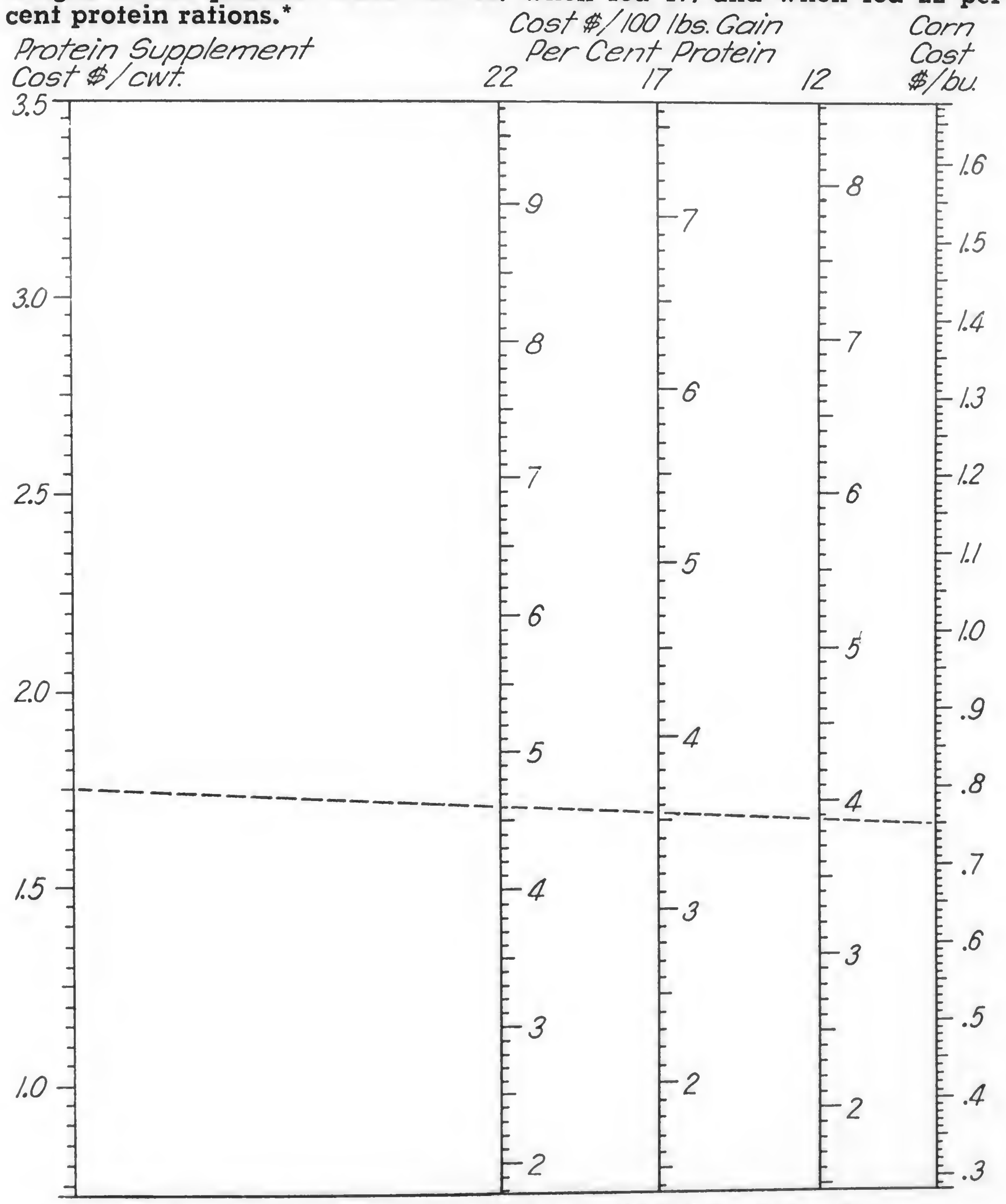
Nomograph 5: For the rapid calculation of the feed cost of 100 pounds gain of pigs from an average initial weight of 75 pounds to an average final weight of 128 pounds, when fed 12, when fed 17, and when fed 22 per cent protein rations.*



*Feeds and quantity required for each 100 pounds gain.

PER CENT PROTEIN IN RATION	22	17	12
Feeds	*pounds*	*pounds*	*pounds*
Corn	133.8	170.4	271.2
Tankage	57.0	31.8	12.4
Soybean oilmeal	57.0	31.8	12.4
Alfalfa meal	10.0	9.8	12.4
Salt	1.2	1.2	1.6
TOTAL	259.0	245.0	310.0

Nomograph 6: For the rapid calculation of the feed cost of 100 pounds gain of pigs from an average initial weight of 130 pounds to an average final weight of 220 pounds, when fed 12, when fed 17, and when fed 22 per cent protein rations.*



*Feeds and quantity required for each 100 pounds gain.

PER CENT PROTEIN IN RATION	22	17	12
Feeds	*pounds*	*pounds*	*pounds*
Corn	155.6	170.4	246.7
Tankage	66.4	31.8	11.3
Soybean oilmeal	66.4	31.8	11.3
Alfalfa meal	12.1	9.8	11.3
Salt	1.5	1.2	1.4
TOTAL	302.0	245.0	282.0

the 12 per cent protein scale will indicate the costs of 100 pounds gain, which are $4.20, $4.39, and $5.15, respectively.

Nomographs 5 and 6 cover costs of gains for pigs from an initial weight of approximately 74 pounds to a final weight of about 128 pounds, and from an initial weight of approximately 128 pounds to an average final weight of 220 pounds, for rations containing 22, 17, and 12 per cent protein.

As shown by the nomographs, from the financial point of view, for pigs between 40 and 70 pounds, the 22 per cent protein ration is most economical; for pigs between 70 and 125 pounds or 80 to 125 pounds, the 17 and 20 per cent rations, respectively, are the cheapest; and for weights between 125 and approximately 200 pounds, the 15 and 17 per cent rations are the least expensive.

These nomographs indicate that under average marketing conditions, where the supplement may be high in price and the corn cheap, the 15 per cent protein ration may produce the most economical gains for pigs during the weight intervals of 80 to 125 and 125 to approximately 200 pounds. Under market conditions, when corn is high in price and protein supplement is comparatively cheap, a higher percentage of protein may give the most economical gains. These nomographs have indicated that the 12 and 10 per cent protein rations are the most expensive.

Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1940, 45, 50-51

11578 P

Manganese as a Possible Factor Influencing the Occurrence of Lameness in Pigs.*

R. C. MILLER, T. B. KEITH, M. A. MCCARTY AND W. T. S. THORP.
From the Pennsylvania State College.

Impairment of the normal movement of the legs frequently occurs in swine. This condition is variously termed lameness, stiffness, posterior paralysis or incoördination, depending on its severity and etiology. A characteristic stiffness occurs in rickets in pigs, a condition in which there is abnormal bone development. Incoördination occurs in pigs as the result of vitamin A deficiency, in which there are degenerative changes in the central nervous system. In addition, however, lameness of one type or another occurs in some instances in which calcium, phosphorus, and vitamins A and D do not appear to be the limiting factors.

In the course of growth studies of pigs conducted by the authors during the past few years, a type of stiffness or lameness has been encountered which appears to be etiologically different from any of the forms described in the literature. This condition occurred in pigs which were fed similar rations, all of which were comprised of yellow corn, tankage, soy bean oil meal, ground alfalfa hay and salt. The pigs were fed individually and indoors but had access to direct sunlight at all times in outdoor paved pens.

The growth of the pigs was good but lameness occurred at about the time they reached a body weight of 150 pounds. The condition was at first characterized by a slightly halting gait, but it slowly progressed in severity until it was only with difficulty that the pigs could rise to their feet. The behavior of the pigs indicated that the condition was painful. Gross manifestations, in addition to stiffness, were enlarged hock joints and crooked legs which occurred even with some pigs which never became stiff. It should be noted that only 30 out of a total of 60 pigs receiving the above treatment were affected with stiffness. During the period of experiment 9 of the unaffected pigs did not attain the weight of 150 pounds, the weight at which stiffness usually occurred.

---

* Authorized for publication on June 8, 1940, as paper No. 974 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

In outward appearance the condition resembled rickets. However, it was not alleviated or affected in any way by the addition of calcium and phosphorus to the ration or by the administration of cod liver oil and irradiated yeast. Subsequent analysis of the ulni and radii of 18 affected pigs showed that the mineral content of these bones was normal, the average value for bone ash being 58.92% on the dry, fat-free basis. Normal values were also obtained for the inorganic phosphorus content of the blood. However, there were certain constantly occurring changes observed in the leg bones which indicate abnormal development. Upon gross examination, the distal extremity of the radius and of the ulna showed enlargement. Roentgenographs of this area of these bones indicate that there is an increased activity of the tissues at the osteochondral junction, this area being definitely enlarged, but there was no apparent irregularity of the zone of ossification and no other changes present other than hypercalcification, thus eliminating the possibility of rickets.

The occurrence of stiffness was first noticed during the feeding of rations which had relatively high mineral contents. These ranged from 6 to 9% and suggest a point of similarity between these rations and those producing perosis in chicks. In view of the reported efficacy of manganese in preventing perosis, the swine rations were analyzed and found to have low manganese contents ranging from .0011 to .0014%.

Manganese sulfate was then added to the swine rations in amounts to supply 50-60 parts per million of manganese. As a result 15 pigs which received the manganese supplemented rations showed no signs of stiffness. Fifty percent of litter and pen mates of these same pigs fed the unsupplemented rations became stiff as usual. However, the addition of manganese to the ration was ineffective in curing stiffness after it had developed.

Hogan[1] has recently presented evidence to show that factors other than manganese may be involved in the prevention of perosis in chicks. The authors do not claim any similarity between perosis in chicks and the type of stiffness in swine described above, but wish to suggest a possible rôle of manganese in the nutrition of swine which apparently involves bone development.

---

[1] **Hogan, A. G., Richardson, L. R., and Homer, Patrick, paper presented at the New Orleans Meeting of the American Institute of Nutrition, March 13, 1940.**

Reprinted from THE JOURNAL OF NUTRITION
Vol 20, No. 6, December, 1940

THE INFLUENCE OF EXERCISE ON THE GROWING RAT IN THE PRESENCE AND ABSENCE OF VITAMIN B_1[1]

N. B. GUERRANT AND R. ADAMS DUTCHER
Department of Agricultural and Biological Chemistry, Pennsylvania State College

FIVE FIGURES

(Received for publication August 12, 1940)

In a former communication from this laboratory (Guerrant, Dutcher and Chornock, '39) it was shown that when carefully selected groups of young rats were used as experimental subjects under comparable conditions of experimentation, less food was consumed, smaller increases in body weight were made and less severe symptoms of vitamin A deficiency developed when the animals were forced to exercise than when permitted to exercise voluntarily or when confined in the usual type of cage. It was shown that, although animals which were forced to exercise exhibited the greatest efficiency of food utilization, they voided the greatest number of fecal pellets, indicating a beneficial effect of exercise on intestinal motility. Furthermore, it was shown that animals, which were exercised voluntarily, and which received daily allotments of vitamin A, were more active physically than were the litter mates which did not receive the vitamin supplement.

The investigation reported at this time concerns itself with similar studies regarding the effect of exercise on vitamin B_1 requirement.

---

[1] Authorized for publication on July 31, 1940, as paper no. 982 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Presented before the division of biological chemistry at the Cincinnati meeting of the American Chemical Society, April 10, 1940.

EXPERIMENTAL

The present investigation consisted essentially of a comparison of the physiological activity and the growth responses of comparable groups of young rats while being maintained under identical conditions, with the exception of the amount of exercise permitted by the type of caging employed and of the vitamin B_1 intake. The experimental equipment was identical with that used in the earlier experiments. With the exception of the following details, the experimental procedure was also similar to that used in the previous investigation.

Healthy rats, 23 to 26 days of age and weighing approximately 52 gm., were used as the experimental subjects. All animals were fed, ad libitum, a vitamin B_1 deficient basal diet which had the following percentage composition: purified casein 18, salt mixture 4, cellu flour 2, sucrose 71, filtered butterfat 3 and cod liver oil 2. Each animal received, daily, 10 µg. of riboflavin and the equivalent of 100 mg. of rice polish concentrate[2] as supplements to the basal diet. Each set of animals was divided into two groups. The animals of one group received the unsupplemented vitamin B_1 deficient diet until they were depleted of their body reserves of vitamin B_1. With the development of the paralytic symptoms characteristic of vitamin B_1 deficiency, each animal of this group was fed 6 µg. of vitamin B_1 daily for a period of 35 days. The second group of animals served as positive controls and, in consequence, each animal received 6 µg. of vitamin B_1 daily throughout the experimental period.

DATA

The data reported at this time have been reduced to group averages and are given in figures 1 to 5 inclusive. In making these tabulations only data from those animals which survived the entire experimental period have been included.

---

[2] Obtained from The Borden Company, Bainbridge, New York. Two hundred grams of this product were dissolved in 750 ml. of distilled water, autoclaved for 4 hours at 15 pounds pressure, cooled, filtered and made up to 1 liter.

DISCUSSION

It was observed that animals in the "forced exercise" group were unable to undergo the desired 1000 cage revolutions daily, during the first few days of the experiment, without becoming exhausted. As a result, the exercise requirement for these animals was less than 1000 revolutions per day, during this period. The number of revolutions, daily, was gradually increased until all animals were meeting the specified exercise requirement of 1000 revolutions per day, by the end of the first week.

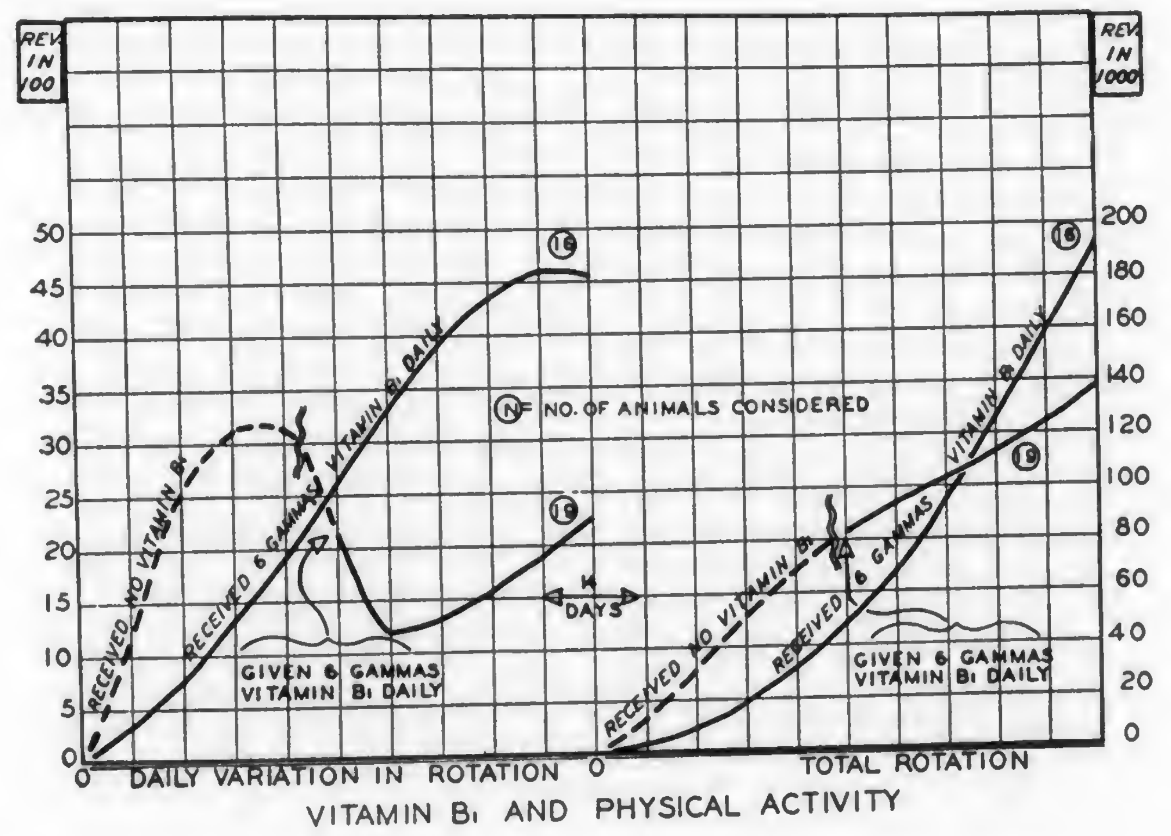


Fig. 1 The mean variation in physical activity of two groups of young rats maintained under comparable conditions in all respects except as to their vitamin B_1 intake.

In the course of these studies a rather significant observation was made in that those animals of the voluntary exercise groups which were depleted of their vitamin B_1 reserve were found to be more active physically, during the first few weeks of the depletion period, than comparable animals, similarly caged, but receiving 6 µg. of vitamin B_1 daily (fig. 1). During the first day or so of the experiment all animals of the voluntary exercise group deported themselves similarly. By the end of the first week, however, it was noted that the animals which were not receiving the vitamin supplement were rotating the wheels of their respective cages more frequently than were the animals which received the vitamin supplement. This condition became even more noticeable as the experiment progressed. By the twenty-first day of the experiment the animals undergoing depletion were making twice as many revolutions as litter mates receiving thiamin, and approximately three times as many revolutions as the animals of the forced exercise group. However, as the onset of the symptoms of vitamin B_1 deficiency became more pronounced, these animals became progressively less active as paralytic symptoms developed. When the animals reached the stage of depletion where physical excitation resulted in spastic paralysis, they were fed the vitamin supplement. In most instances, it was surprising to observe that such animals continued to become even less active for several days after the supplementary feeding was begun. Usually (about the fourth day of the supplementary feeding of 6 μg. of vitamin B_1 daily), these animals began to regain some of their inclination to exercise and continued to do so until the end of the experiment. However, at the end of the experiment, the average daily activity of the rejuvenated animals was considerably less than the daily activity of similar animals which had received the vitamin from the beginning of the experiment. A possible explanation for this may be found in the relationship of vitamin intake to the weight of the experimental animals. This observation would appear to bear some significance in any attempt to estimate the optimal requirement of the rat for vitamin B_1. In this regard, it appears from the data presented in figure 1, that an intake of 6 μg. of vitamin B_1 is insufficient to maintain the growing rat (50–120 gm.) in a state of nervous stability and that this inadequacy becomes even more pronounced as the weight of the rat increases. Therefore, if such is the case, it is to be expected that the smaller animals receiving 6 μg. of vitamin B_1 daily will be found to be more stable physically than larger animals receiving similar amounts of the vitamin. A further suggestion that the quantity of vitamin B_1 used in these studies (6 μg. daily) was not adequate to maintain the growing rat in a state of nervous equilibrium is found in the fact that the animals used in these studies were far more active physically than were the animals used in our previous studies where a basal diet containing 8% of dried yeast was fed.

An inspection of the data presented in figures 2 and 3 reveals that exercise, under the conditions in which it was employed in these studies, had a measurable effect on the growth rate of young rats irrespective of whether or not the animals were receiving the vitamin B_1 supplement. Those animals of the voluntary exercise group, which were most active in rotating their cage wheels made smaller increases in body weight than did the less active animals. This observation was most striking during the depletion period, where the animals of the voluntary exercise group showed a smaller initial increase in body weight (8 gm.) and in turn lost a greater percentage of this weight (32%) before developing the paralytic symptoms. In fact the mean weight of the animals of this group at the end of the depletion period was 41 gm., or 11 gm. less than their initial weight (when placed on experiment). The maxi-

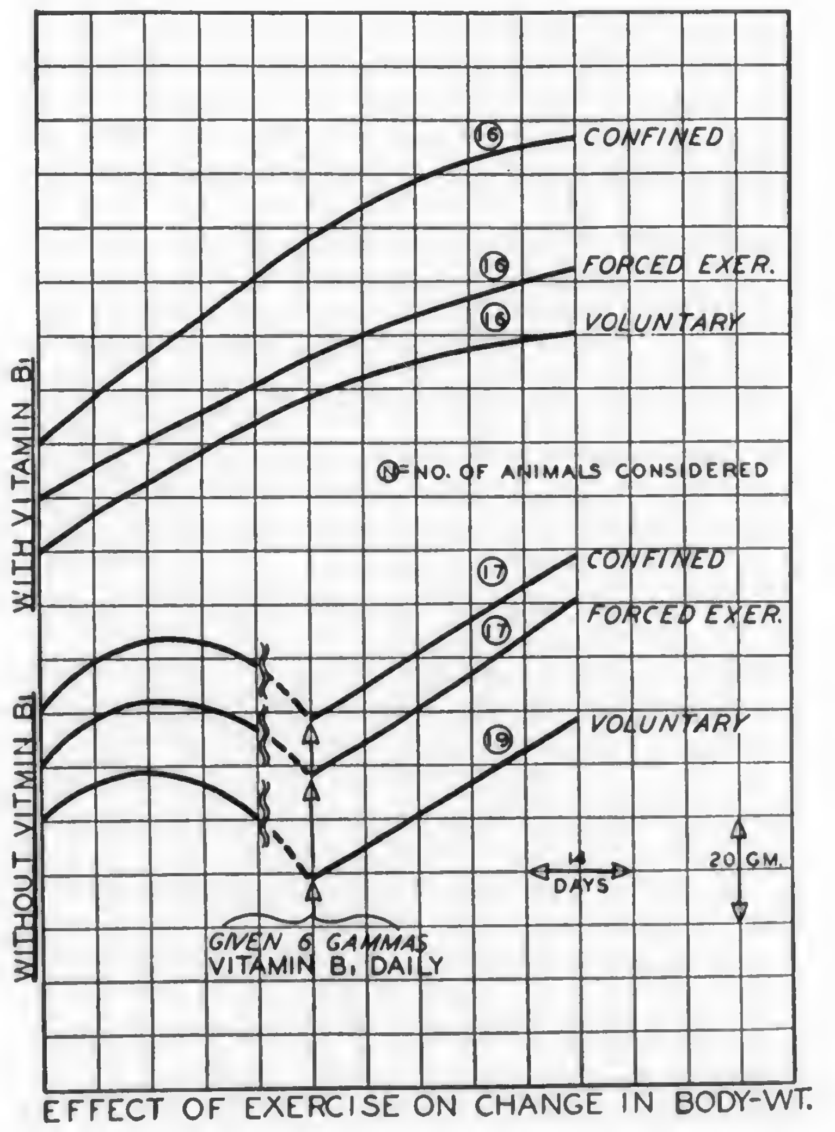


Fig. 2 The effect of different amounts of exercise on the growth rate of young rats while being maintained under comparable dietary conditions except as to their vitamin B_1 intake.

mum gains in weight for the three groups of animals (voluntary exercise, forced exercise, and confined) during the depletion period were 8, 13, and 14 gm. respectively, while the subsequent losses in weight for the same groups of animals were 20, 16, and 15 gm. respectively. The mean increase in body weight following the supplementary feeding of vitamin B_1 was found to be quite comparable for the three conditions of exercise. These values, when given in the above order of groups, were 29, 33, and 30 gm. respectively. In this connection, it should be borne in mind that the activity of the animals of the voluntary exercise group, during this time, was considerably less than during the depletion period, and probably more comparable to the activity of the forced exercise animals. In the instance of the animals which received the vitamin supplement from the beginning of the experiment, those of the confined group grew at a considerably faster rate than did the animals of either of the other two groups (voluntary and forced). While it is possible that the nature or the composition of the body weight increments produced under the different conditions of exercising may have been quite different, no data are at hand to substantiate this view. However, later studies

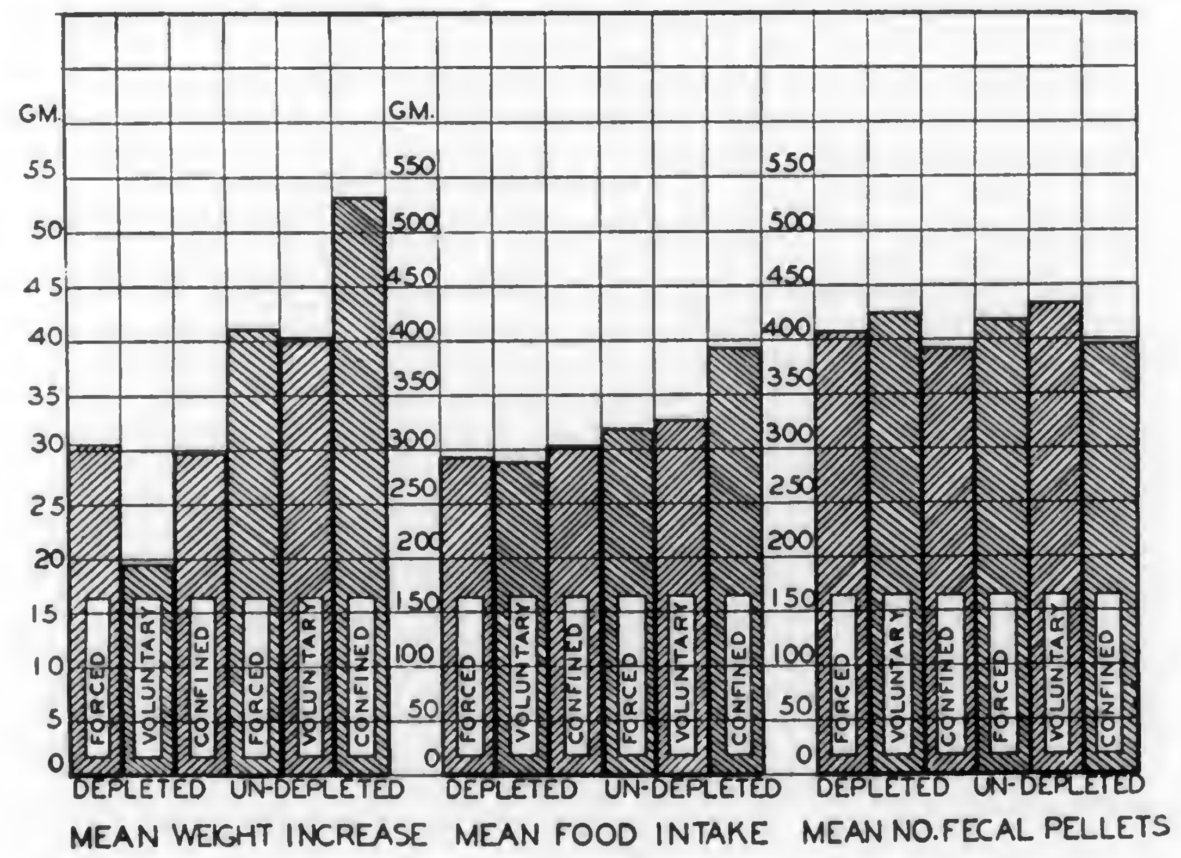


Fig. 3 The relative effects of exercise on the mean body weight increase, the mean food intake and the mean number of fecal pellets voided by young rats while receiving diets identical in all respects except in their vitamin B_1 content.

on the chemical composition of body tissues may shed further light on this problem.

From the standpoint of food consumption, the various degrees of exercise did not appear to be an important consideration (fig. 3). Although under both conditions of vitamin supplementation, the animals of the confined groups consumed the greatest amounts of food during the experimental period, the relationship of food intake to body activity is not clearly defined in all instances. It is evident from these data that the matter of growth is a complex phenomenon which is influenced by a number of factors, among which are food intake and exercise.

A similar consideration of the fecal output of the various groups of animals (fig. 3) reveals that there was no great variation in the number of fecal pellets voided by the respective groups of animals during the entire experimental period. However, it seems significant that in both instances the animals of the voluntary exercise groups voided the greatest number of fecal pellets while the animals of the confined group voided the smallest number of fecal pellets. A tabulation of the data regarding the effect of exercise on the weight of the fecal matter, the percentage of fecal matter (relative proportion of food excreted as fecal matter) and the relative sizes of the fecal particles (fig. 4) reveals some significant differences between the animals of the three experimental groups in these respects.

The animals of the confined groups produced the greatest weight of fecal matter, the greatest percentage of fecal matter and the largest fecal pellets (as indicated by weight), while the animals which exercised most strenuously (voluntary exercise group) produced the least fecal matter, the smallest percentage of fecal matter and the smallest fecal pellets. While some of these differences may not be significant, the data clearly indicate a beneficial effect of exercise on fecal elimination. Another interesting observation relates to the fact that under all conditions of exercise, those animals which received vitamin B_1 utilized their food more efficiently (lower percentage of fecal matter) than did similar animals not receiving the vitamin supplement.

The data given in figure 5 demonstrate the effect of exercise on the average time required for the animals of the various

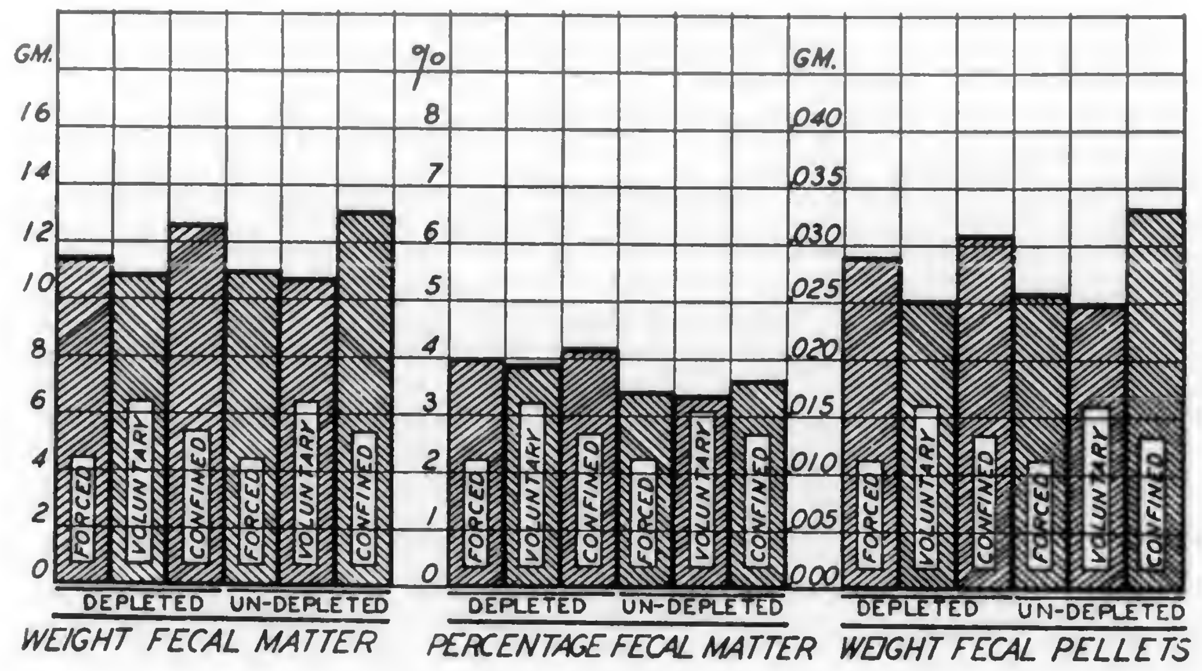


Fig. 4 The effect of exercise on total weight of fecal matter, percentage of fecal matter and mean weight of fecal pellets voided by young rats while receiving diets identical in all respects except as to the amount of the vitamin B₁ supplement.

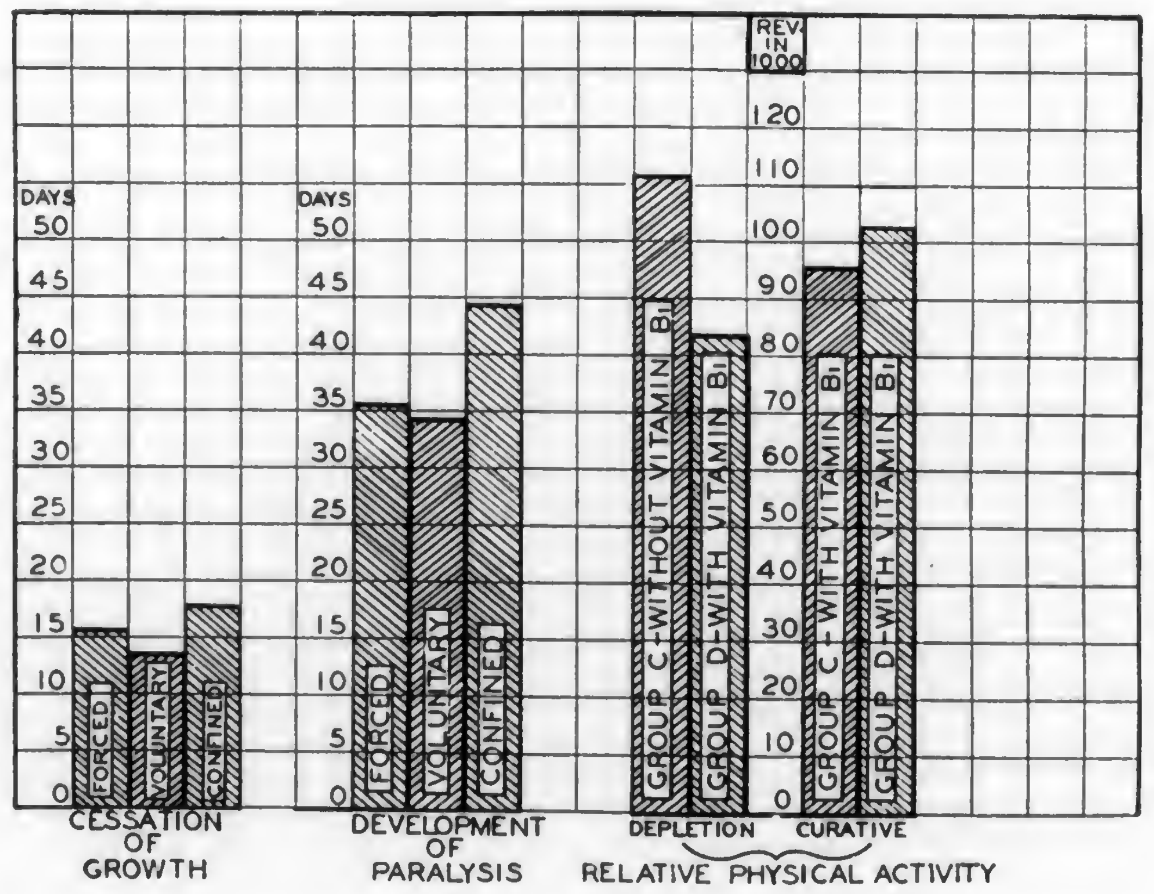


Fig. 5 The effect of exercise on the time required for the cessation of growth and for the development of the paralytic symptoms among groups of young rats receiving a diet deficient in vitamin B₁. The figure also shows the relative activity (as indicated by the rotation of the cage wheel) of two comparable groups of young rats, of which the animals of one group (group D) received daily 6 μg. of vitamin B₁ throughout the experimental period, while the animals of the other group (group C) received no added vitamin B₁ during the depletion period but received daily 6 μg. of this vitamin during the last 35 days of the experimental period.

groups, undergoing depletion, to cease growing. It will be noted that the animals of the voluntary exercise group ceased growing after about 13.5 days on the deficient diet, while the animals of the forced exercise and of the confined groups required 16 and 17.6 days, respectively. Corresponding data relative to the average time required for the development of the paralytic symptoms were equally significant. The animals of the voluntary exercise group manifested the characteristic paralytic symptoms on the thirty-fourth day of the depletion period, while the animals of the forced exercised and of the confined groups required 36 and 44 days respectively. These findings clearly indicate that young rats undergoing vigorous exercise have somewhat greater requirements for vitamin B₁ than do similar animals which undergo little or no exercise. This is in agreement with the data obtained by Cowgill and co-workers ('31) in their studies with dogs but is not in agreement with our previous findings regarding the influence of exercise on the vitamin A requirement of the growing rat. However, it seems highly probable that the latter variation may be due to differences in the physiological function of the two vitamins. It appears reasonable to postulate that the symptoms of vitamin A deficiency are accentuated by the accumulation of toxic substances in the body of the experimental animal due to poor elimination. Therefore, any factor, such as exercise, which increases elimination may bring about a delay in the development of such symptoms. On the other hand, vitamin B₁ which is known to play an important role in the complicated phenomena associated with cellular oxidation and muscle contraction may be required in increased amounts during periods of vigorous exercise. In fact the data obtained in these studies indicate that the vitamin B₁ requirement of the rat is more dependent on the rate of metabolism than it is on the caloric intake.

SUMMARY

Experiments conducted with carefully selected groups of young rats as experimental subjects have yielded data which demonstrate that the vitamin B₁ requirement of these animals, as indicated by the time required for the cessation of growth and for the development of the paralytic symptoms, is increased by physical exercise. These findings are not in agreement with previous findings concerning the influence of exercise on the vitamin A requirement of the growing rat. A possible explanation of this variation may be found in the differences in the physiological function or functions of the two vitamins.

In the instance of the animals receiving no vitamin B_1, increased exercise resulted in less food consumption, less growth, early development of paralytic symptoms and the elimination of a greater number of fecal particles. With the animals receiving 6 μg. of vitamin B_1 daily, increased exercise resulted in less growth and in the elimination of a greater number of fecal pellets. During the first 4 weeks of the test period, those animals which were permitted to exercise voluntarily were more active physically while not receiving vitamin B_1 than were comparable animals, similarly caged, but receiving vitamin B_1. However, during the latter stages of vitamin B_1 depletion, physical activity among these animals declined to a minimum of about one-third of its previous value. This decline in physical activity continued for approximately 6 days after the administration of daily dosages of 6 μg. of vitamin B_1. There was a steady resumption of activity by these animals during the last 4 weeks of the experimental period, as the result of the administration of the vitamin. Definite nervous instability resulting from vitamin B_1 deficiency was indicated, during the early part of the depletion period, by the increased tendency of the animals to exercise.

LITERATURE CITED

COWGILL, G. R., H. A. ROSENBERG AND J. ROGOFF 1931 Studies in the physiology of vitamins. XVI. The effect of exercise on the time required for the development of anorexia characteristic of lack of undifferentiated vitamin B. Am. J. Physiol., vol. 98, pp. 589–594.

GUERRANT, N. B., R. ADAMS DUTCHER AND FRANCIS CHORNOCK 1939 The influence of exercise on the growing rat in the presence and absence of vitamin A. J. Nutrition, vol. 17, p. 473.

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 131, No. 2, December, 1940

THE EFFECT OF HIGH PRESSURE TREATMENT ON THE PHYSIOLOGICAL ACTIVITY OF INSULIN

R. B. DOW, J. E. MATTHEWS, JR. AND W. T. S. THORP

From the Departments of Physics, Agricultural and Biological Chemistry and Animal Husbandry of The Pennsylvania State College, State College, Pennsylvania

Received for publication July 15, 1940

Many of the chemical and physical properties that affect the physiological activity of insulin have been summarized by Jensen (1). The writers are not aware, however, of any study of the effect of hydrostatic pressure on the physiological activity. Several researches have shown that high pressure is an effective means of controlling many reactions of biological and bio-chemical interest. Bridgman (2) in 1914 reported that egg albumin is coagulated by high pressure and with Conant (3) in 1929 found that carboxyhemoglobin is denatured under similar conditions. More recently, Bassett and his collaborators (4) have reported the effects of high pressure on several bacteria, vaccines, and enzymes but the data are of a preliminary nature and few conclusions can be made. Investigations in the high pressure laboratory of the Department of Physics of this College by Dow and Matthews (5) and by Matthews, Dow and Anderson (6) have demonstrated that denaturation of proteins by high pressure results in their deactivation. The extent of denaturation depends on the magnitude of the pressure and the time of exposure, as well as on several chemical factors, for example, the pH of the solution.

Since it is generally agreed that insulin is a typical protein, it would be of interest to study its denaturation at high pressure in relation to its physiological activity.

EXPERIMENTAL METHOD. Insulin solutions from two sources were used in these experiments. Lilly's U-20 Iletin was used in the major part of the investigations and some study was made with amorphous insulin (17 units per mgm.) from the Banting Institute.

The apparatus for hydrostatic pressure treatment is similar to that used extensively by Bridgman (7) and by the senior author in investigating various physical and chemical behavior at high pressure. Initial pressures produced by a hand-pump are further intensified by a hydraulic press that generates high pressure in the test chamber. Pressure can be increased or reduced at such a slow rate that there is no sensible change of temperature during the process. A thermostated bath can be placed about the test chamber to keep it at constant temperature, although in these experiments it was not considered essential and the treatments were applied at room temperature.

The sample of insulin was placed in one of two types of containers. Flexible tooth paste tubes lined with paraffin were first tried as containers. The tubes were used by soldering the lower ends, filling with the test sample, and then sealing by means of the threaded cap. Consequently the sample was separated mechanically from the kerosene that transmitted pressure in the test chamber. A brass tube, lined similarly with paraffin and closed at one end, served as the other type of container. As the samples did not completely fill the tube, paraffin oil was poured in to cover the insulin with a layer of about one inch in depth. Other experiments had shown previously that good separation of liquids was obtained in this way and that similar results were found irrespective of the nature of the container. This is similar to the experience of Basset (4). Both containers were used indiscriminately in these experiments but most of the tests were made with the latter type.

The physiological activity of both normal and pressure treated insulin was measured by the percentage lowering of blood sugar in rabbits that were given standard doses, and doses of the pressure treated samples that were considered the equivalent of standard doses. A standard solution was considered to have 2.5 physiological units per cubic centimeter of solution and was made up with U-20 Iletin in physiological salt solution. A standard dose was taken as 1 cc. of solution that contained 2.5 units per 2 kgm. of body weight. The pressure treated samples were made up in the same way by using the same quantity of treated insulin in physiological salt solution. For the pressure treatment, however, the Iletin was used as it was received from the maker but the amorphous insulin was made up in physiological salt solution to contain 140.1 units per cubic centimeter. While the onset of convulsions, as well as the intensity and length of them, were observed they were not considered sufficiently reliable measure of the insulin activity to be recorded in this paper. One reason for this was the wide variation of the convulsive level in the rabbits.

The animals were not ideal for the purposes of the experiments. They were of mixed breed and evidently varied considerably in physical stamina. Several of their ears were unsuitable for bleeding. Two females and ten males were used in the experimental procedure. Of the former, rabbit 7 unexpectedly gave birth prematurely to a litter during one of the tests. She had been used previously several times, giving consistent behavior as can be judged by the data. Culhane (8) has reported that females having a litter are generally unsuited for tests. It is thus surprising to observe such consistent results with a pregnant rabbit. The ears of the other female were so unsuited for bleeding that she could not be used after a preliminary experiment.

The method of "cross-test" advocated by Culhane (8) was used in the experiments. By this procedure one group of animals is given the standard preparation and another group the test sample. This method was followed twice a week using four animals for each test but each animal was not used more than once a week in most cases. Two rabbits were usually given the standard insulin dose and two others the pressure treated sample. The tests were carried out over a period of five weeks.

The diet prescribed for the rabbits consisted of hay and oats. The rabbits were without food 24 hours prior to the experiments. During this period, as well as during the tests, they were allowed an ample amount of water. After inoculation samples of blood were drawn from the ears at $1\frac{1}{2}$, 3 and 5 hours, respectively. These together with the blood sample that was always taken before inoculation were sufficient to allow the change in blood sugar to be followed with some accuracy. The micromethod of Folin and Malmros (9) that requires only 0.1 cc. of blood was used to determine the blood sugar content.

TABLE 1

Percentage sugar decrease in rabbits inoculated with pressure treated and untreated iletin

ANIMAL	PER CENT CHANGE UNTREATED ILETIN	PER CENT CHANGE WITH PRESSURE TREATED ILETIN					AVERAGE PER CENT CHANGE OF PRESSURE TREATED ILETIN	PER CENT DIFFERENCE TREATED-UNTREATED ILETIN
		1 5,000 kgm./cm^2.	2 5,000 kgm./cm^2.	3 10,000 kgm./cm^2.	4 10,000 kgm./cm^2.	5 10,000 kgm./cm^2.		
1	45		30	17	46	43	32	−13
2	No results							
3	71	71	73 Died				72	+1
4	47		31	36			34	−13
5	55*	65	74	51	Died		63	+8
6	56		51	43	39		44	−12
7	55*		59	45	47		50	−5
8	57*			38 53**	64	57	53	−4
9	60	64	66	40	69	59	60	0
10	58	70	77 Died				74	16
11	63				46	62	54	−9
12	No results							
Average								−5

* Two determinations differing by 1%.
** Amorphous insulin treated for 3 hrs.

Data and results. The experimental results summarized in table 1 were obtained with Iletin, except in one case as indicated. The maximum percentage decrease in blood sugar level is recorded for normal insulin and pressure treated samples at 5,000 and 10,000 kgm/cm^2. Two insulin samples were treated at 5,000 kgm/cm^2. The exposures, tests 1 and 2, were for 3 and 15 hours, respectively. Three treatments at 10,000 kgm/cm^2, tests 3, 4 and 5, were made for 15, 23 and 41 hours, respectively. To determine the maximum percentage decrease in sugar level, the four sugar determinations, measured as the number of milligrams per 100 cc. of blood, were plotted graphically and the maximum decrease was read from the resulting curve in each case. This method was adopted since it was found that the curves were always similar but not of a simple nature. Since they showed a definite relation in respect to time, a simple average of sugar determinations during the 5 hour period would have little significance.

While it can be shown that the average of the percentage decreases for the treated samples is about 5 per cent less than the average for untreated insulin, this difference does not prove necessarily that the physiological activity of insulin is reduced by pressure treatment. Considering that the animals used in this study were not the best for the purpose, and the fact that relatively few were available for the tests, it would appear reasonable to assume that 5 per cent represents a likely error in these experiments. On this basis the writers are inclined to state that pressure treatment had no effect on the physiological activity of insulin.

Although the pressure treatments did not change the appearance of Iletin, it was realized that with stronger concentrations of insulin, and particularly with insulin that contained no preservative, pressure might produce coagulation as had been observed previously (6) in the case of pepsin. To verify this the amorphous insulin in physiological salt solution was subjected to a pressure treatment of 10,000 kgm/cm^2 for 3 hours. In this case, however, the treatment did produce coagulation that was clearly visible. The precipitate appeared similar to the appearance of egg albumin, or any other typical protein, such as pepsin, when it is coagulated by heat. The precipitate was carefully mixed with additional salt solution to a dilution corresponding to the previous doses and then injected into a starved rabbit. The animal was no. 8 that had been used in the previous experiments. The maximum percentage decrease in sugar was found to be 53 per cent, a value close to that found for the animal previously (table 1). The physiological activity of amorphous insulin was clearly not affected by the fact that coagulation resulted from the pressure treatment.

Van Slyke amino nitrogen determinations were made on both the control solution of amorphous insulin and the pressure treated sample with the result that no change in amino nitrogen was detected. Consequently there is no evidence of hydrolysis by pressure.

Discussion. It is well known that insulin loses its physiological potency when denatured by heat. In this respect it behaves similarly to many other proteins. The present experiments show, however, that while insulin may be denatured by pressure, as indicated by coagulation in concentrated solutions, the treatment does not reduce its physiological activity. Evidently denaturation caused by pressure must be fundamentally different from that brought about by heat. Since when applying pressure to a sample the rate of compression can be so low that there is no appreciable rise of temperature, the process can be said to be carried out isothermally. It is important to note that during such a compression more energy flows out of the sample in the form of heat than is put in by the work of compression. This is easily understood when it is recalled that the attractive forces in the liquid must do work during the compression. It has been shown previously (6) that by compressing water isothermally to a pressure of 1,000 kgm/cm.^{2}, about seven times more energy flows out of the water than is put in by the compression. When it is remembered that in denaturing by heat energy must always flow into the sample, as indicated by the temperature rise, it is evident that in pressure denaturation the physical process insofar as energy is concerned, is decidedly different. This appears to be a significant factor that has not been studied thoroughly. It suggests many possibilities for future study since it may involve some reaction within the protein molecule that is not known at present.

The recent theory of Wrinch (10, 11, 12) on the structure of proteins has given impetus to speculations on the nature of the changes that take place in denaturation and hydrolysis. According to this theory denaturation takes place when the cyclol bonds are broken, but the polypeptide chains remain uneffected. A breaking of a peptide link, however, involves a reaction with another molecule and is to be associated with hydrolysis. According to this interpretation, the pressure denaturation observed in these experiments broke the cyclol bonds in the insulin molecules with the result that the polypeptide chains became the predominating type of structure. No change in amino nitrogen would be expected unless hydrolysis occurred. Since no change of activity has been observed in these experiments, it would appear that the physiological properties of insulin are bound up in some manner with the polypeptide linkage in the molecule, denaturated or otherwise. The possibility of associating the specific physiological properties of insulin with its component amino acids has been discussed by Jensen (13).

The authors wish to acknowledge the donation of the Iletin by Dr. W. B. Peck of the Lilly Research Laboratories and the amorphous insulin by Professor W. R. Franks, of the Banting Institute. They are indebted to Dr. A. K. Anderson of this college for his kindness in putting his equipment at their disposal.

SUMMARY

The physiological activity of insulin is unaffected by long exposure to pressure of the magnitude of 10,000 kgm/cm^2. Activity was measured by observing the percentage decrease of blood sugar level in rabbits.

Evidence that pressure produced denaturation is given by the coagulation of insulin. Coagulation does not affect the physiological activity. The absence of any change in amino nitrogen content of the insulin has been taken to mean that pressure does not produce hydrolysis under these conditions.

It is suggested that the results of these experiments indicate that the physiological activity of insulin is associated with its polypeptide or amino acid linkages, rather than with any particular bonding or grouping of the chains such as might exist in the undenaturated state of the molecule.

REFERENCES

(1) Jensen, H. F. Insulin, its chemistry and physiology. The Commonwealth Fund, New York, 1938.
(2) Bridgman, P. W. J. Biol. Chem. **19**: 511, 1914.
(3) Bridgman, P. W. and J. B. Conant. Proc. Nat. Acad. Sci. **15**: 680, 1929.
(4) Macheboeuf, M., J. Basset and G. Levy. Ann. de Physiol. **9**: 713, 1933.
(5) Dow, R. B. and J. E. Matthews, Jr. Phil. Mag. **27**: 637, 1939.
(6) Matthews, J. E., Jr., R. B. Dow and A. K. Anderson. J. Biol. Chem. **135**: 697, 1940.
(7) Bridgman, P. W. Physics of high pressure. MacMillan, New York, 1931.
(8) Culhane, K. Quart. J. Pharmacol. **1**: 517, 1928.
(9) Folin, O. and H. Malmros. J. Biol. Chem. **83**: 115, 1929.
(10) Wrinch, D. M. Phil. Mag. **25**: 705, 1938.
(11) Langmuir, I. and D. M. Wrinch. Nature **143**: 49, 1939.
(12) Wrinch, D. M. and I. Langmuir. J. Am. Chem. Soc. **60**: 2247, 1938.
(13) Reference 1, pp. 66–69.

Essentials of Nutrition. *H. C. Sherman* and *C. S. Lanford*, Columbia University. First Edition. The Macmillan Company, New York City, 1940. x + 418 pp. 34 figs. 10 × 17 cm. $3.50.

The purpose of this book is to "offer its readers a thoroughly adequate and up-to-date view of the essentials of nutrition." The book is written for the reader who has had no previous training in science. Anyone who has endeavored to interpret biochemical research for lay audiences will appreciate the admirable manner in which the authors have handled this difficult task. Chemical formulas and complex chemical definitions and explanations are conspicuous by their absence.

The book, which evidently is designed for the freshman college student, contains 21 chapters covering most of the usual topics of interest to students of nutrition. These are treated, however, from the viewpoint of practical application. A few representative chapters are as follows: I. The Nutritional Improvement of Life; V. How to Meet the Energy Need and Have the Body Weight You Want; VI. How to Meet the Need for Protein; XI. Food Costs and Food Values; XXI. How to Make Nutritional Knowledge More Effective, etc. Each chapter is followed by thought-provoking "exercises" and "suggested readings." In the opinion of the reviewer, most of the latter cannot be read to advantage by the type of reader for which the book is intended. Other chapters deal with the essential facts of digestion, food composition, function of food ingredients, mineral elements, vitamins, etc. Naturally, discussions of metabolic changes are omitted.

The appendix is divided into five parts giving, in greater detail, information to supplement the text. An adequate subject index comprises the last 15 pages.

The reviewer feels that the discussion of statistical methods could be omitted without detracting from the use of the book. The style is simple and understandable and will appeal to those who have had no formal scientific training; the book should be in every high-school library. It is the hope of the reviewer, however, that college teachers will not be tempted to use this excellent book as an excuse for even less formal chemical training of college students who are contemplating a college course in nutrition.

R. Adams Dutcher

Pennsylvania State College
State College, Pennsylvania

Reprinted from THE JOURNAL OF NUTRITION
Vol. 21, No. 4, April, 1941

THE OPTIMUM LEVEL OF PROTEIN INTAKE FOR THE GROWTH AND FATTENING OF SWINE[1]

R. C. MILLER AND T. B. KEITH

Department of Agricultural and Biological Chemistry and Department of Animal Husbandry, Pennsylvania State College, State College

(Received for publication October 26, 1940)

INTRODUCTION

The choice of the level of protein when expressed as a percentage of the ration to be fed to pigs, presents a major problem in the nutrition of this species. Contributing factors to the complexity of this problem are: (a) the relatively rapid growth rate of swine, which exceeds that of any other class of livestock, (b) possible decreasing requirements for protein during the progress of growth, and (c) economic considerations, especially those concerning the relatively high price of protein supplements.

Carroll and Mitchell and associates ('32, '34, '35, '36, '37) have investigated by means of growth and nitrogen balance studies the question of the changing requirements of pigs at different stages of growth, and have shown that for pigs weighing less than 100 pounds the ration should contain more than 17% of a protein mixture obtained from corn, tankage, soybean oil meal or linseed oil meal, and alfalfa meal. For pigs weighing 150 pounds, 17% of protein was found to be adequate and 15% was enough for pigs weighing 175 to 200 pounds. For pigs weighing 40 to 100 pounds, protein levels up to and exceeding 22% were indicated, by nitrogen balance studies, as being necessary for maximum growth.

---

[1] Presented in part before the Division of Agricultural and Food Chemistry at the Boston Meeting of the American Chemical Society, September, 1939.

Authorized for publication on October 23, 1940, as paper no. 996 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Recently Carroll and Burroughs ('39) in a preliminary report of dry-lot group-feeding trials have shown that young pigs must receive a higher percentage of protein than is sufficient for heavier pigs, and their data suggest that the ration of 50-pound pigs should contain at least 18 to 20% of protein or perhaps more. Between the weights of 75 and 100 pounds their data do not indicate any advantage in gains for more than 15% of protein in the ration, and 12% of protein is indicated for pigs weighing from 150 to 200 pounds.

Woodman, Evans, Callow and Wishart ('36) have concluded from the results of individual feeding trials and group feeding tests that a feeding treatment providing 12% of fish meal (17.48% crude protein) from weaning to 90 pounds live weight, 10% of fish meal (15.93% crude protein) from 90 to 150 pounds, and 5% of a mixture of equal parts of meat meal and ex. soya-bean meal (12.25% crude protein) in the final period will supply all of the digestible protein for the maximum rate of growth permitted by the net energy content of the ration. Higher amounts of protein in the ration were of no benefit and actually depressed the rate of live weight increase in some cases.

From these reports it would appear that, while the minimum protein levels which will produce good growth in pigs are fairly well recognized, the relative value of higher levels of protein intake is not well established. Experiments dealing with the general problem of how much protein it is nutritionally advisable to feed to growing pigs have been conducted by the authors since 1936 and this report presents some of the results of this research.

EXPERIMENTAL

Four experiments were conducted. The number of pigs used in each experiment, the protein levels compared, and the approximate intervals of body gain studied are given in table 2.

The composition of the rations is given in table 1. Number 2 yellow corn was used throughout the experiments. The tankage was a 36% protein, steam rendered by-product of a local abattoir and was relatively high in bone content. The soybean oil meal used in these experiments was processed by the expeller method from beans of the Illini, Manchu, Dunfield, Richland and Mandell varieties. The alfalfa meal was produced from local sun-dried hay by passing it through a hammer mill. One-half of 1% of salt and 1% of ground limestone were included in the rations of experiment 1. The limestone was omitted in experiments 2, 3 and 4, since the chemical analysis indicated that the additional calcium was not necessary and did not influence the calcium-phosphorus ratio to any marked extent. In addition direct sunlight was available in all of the experiments.

TABLE 1

Composition of rations

INGREDIENT	EXPERIMENT 1		EXPERIMENT 2		
	PER CENT PROTEIN		PER CENT PROTEIN		
	10	15	15	20	25
	%	%	%	%	%
Yellow corn	93	76	76.5	57.5	42.5
Tankage	1	15	15	20	18
Soybean oil meal	1	4	4	18	35
Alfalfa meal	4	4	4	4	4
Salt	0.5	0.5	0.5	0.5	0.5
Ground limestone	0.5	0.5	....	....	....

INGREDIENT	EXPERIMENT 3			EXPERIMENT 4		
	PER CENT PROTEIN			PER CENT PROTEIN		
	12	17	22	17	22	27
	%	%	%	%	%	%
Yellow corn	87.5	69.5	51.5	65.5	45.5	36.5
Tankage	4	13	22	12	22	33
Soybean oil meal	4	13	22	12	22	20
Alfalfa meal	4	4	4	10	10	10
Salt	0.5	0.5	0.5	0.5	0.5	0.5

The rations were designed to provide the different levels of protein intake indicated in table 1. No determinations were made of the digestibility of the protein or of its biological value. Rather the scope of these experiments was limited to a study of the relative values of the gross levels of protein intake for pigs of different ages, it being felt that a study of the influence of the level of protein intake and of the stage of development of pigs, on the digestibility and biological value of the rations constituted a separate problem for later study. However, due to the similarity in the constitution of the various rations it is not thought that their digestibilities are markedly different.

Pigs of the Poland China, Chester White, Duroc Jersey and Berkshire breeds were used. They were housed in an experimental feeding house provided with a concrete floor and having outside concrete runways. They were fed as matched pairs in experiment 1 and as matched triplets in the other trials. The experimental pairs or triplets were selected to be of the same breed, sex, age and of similar weight, and members of each pair or triplet were fed the same daily quantity of feed. The amount of feed allotted daily to each member of a pair or triplet was determined by the feed consumption of the pig in each pair or triplet which consumed the smallest quantity of feed; hence, the feed intake was probably restricted to some extent for one member of each pair and two members of each triplet. The pigs were fed three times daily in individual stalls especially constructed for these experiments. They were weighed weekly, and the initial and final weights as given in table 2 are the averages of weights taken on 3 successive days.

RESULTS AND DISCUSSION

Table 2 is a summary of the results. An analysis by variance was made on the data for individuals for the average daily gains and for the feed requirement for 100 pounds of body gain. There are also given the differences between means which are required to show significance at the 5% point of probability.

In experiment 1 the gains for all of the pigs on the 15% level of protein exceeded those of the pigs on the 10% level and were attained with less feed per unit of gain.

The data for experiment 2 covering the growth span from 84 to 200 pounds, also have been broken down to cover other intermediate weight increments. Gains and food efficiency were less for pigs on the 25% protein level in a number of instances. The comparison for the weight interval 127 to 200 pounds was made with only seven pigs at each level of protein intake, there being only seven triplicates which attained the required initial weight of 127 pounds in comparable time intervals. Significant differences were not obtained with respect to gains or feed requirement between the 15 and 20% protein levels, both of which are indicated as better than the 25% level.

Lighter pigs weighing 40 pounds were used in experiment 3. The 12% protein level proved unsatisfactory for these pigs. On this level three pigs failed to gain in a manner satisfactory enough to be included in the comparison, and the feed requirement for the remaining nine pigs was relatively high as was the variation between individuals. Consistent results were obtained favoring the 17% protein level, although on the 22% protein level similar results were obtained in some of the comparisons. The failure of individual pigs to attain the required weights in time intervals to give valid comparisons necessitated making comparisons, in some cases, between unequal numbers of individuals on the respective protein levels. The number of individuals used on any one level is, therefore, some indication of the merit of that particular protein level.

A summary of the results shows that neither an increased rate of body gain nor increased feed efficiency results from a 25% protein level of feeding as compared with either a 15 or 20% level. A 10% protein level was shown to be inferior to a 15% level as judged by body gains and by feed efficiency. Results obtained with 12 and 22% protein levels showed no significant differences favoring either level with respect to growth. Both of these levels, however, seemed to be less satisfactory than the 17% protein level judging by both the average daily gains of the pigs and the efficiency of their food utilization.

TABLE 2

Summary of average weights, body gains and feed requirements of pigs fed rations of different protein content

NUMBER OF PIGS	AMOUNT OF PROTEIN IN RATION	AVERAGE AGE AT BEGINNING	LENGTH OF EXPERIMENTAL PERIOD	INITIAL BODY WEIGHT	MEAN AVERAGE DAILY GAIN	MEAN AVERAGE DAILY RATION	FEED FOR EACH 100 LBS. GAIN
	%	*days*	*days*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Experiment 1 — 130 to 180 lbs.							
10	10	222	39	131	1.08	4.66	447
10	15	193	39	129	1.43	4.65	326
			standard error		.10		17
	difference required to be significant				.31		58
Experiment 2 — 84 to 127 lbs.							
13	15	136	35	82	1.34	3.53	269
13	20	121	31	85	1.39	3.42	252
13	25	125	33	86	1.28	3.58	284
			standard error		.05		15
	difference required to be significant				.16		45
	84 to 156 lbs.						
13	15	136	53	82	1.39	3.88	281
13	20	121	50	85	1.47	3.83	265
13	25	125	54	86	1.31	3.89	300
			standard error		.05		16
	difference required to be significant				.13		48
	127 to 200 lbs.						
7	15	166	39	128	1.76	4.71	268
7	20	154	46	128	1.73	4.58	265
7	25	162	45	127	1.49	4.60	316
			standard error		.06		19
	difference required to be significant				.19		59
	84 to 200 lbs.						
13	15	136	77	82	1.55	4.28	276
13	20	121	77	85	1.55	4.28	276
13	25	125	77	86	1.38	4.29	314
			standard error		.04		12
	difference required to be significant				.12		33
Experiment 3 — 40 to 74 lbs.							
9	12	77	76	40	.45	1.56	375
12	17	70	72	38	.50	1.43	301
12	22	73	70	41	.51	1.31	289
			standard error		.03		29
	difference required to be significant				.10		85
	74 to 156 lbs.						
7	12	156	73	73	1.14	3.25	285
12	17	144	58	75	1.43	3.28	229
11	22	145	69	75	1.18	3.29	274
			standard error		.04		11
	difference required to be significant				.15		33
	156 to 213 lbs.						
4	12	213	35	155	1.68	4.64	283
11	17	202	33	156	1.83	4.61	257
6	22	214	38	155	1.52	4.66	309
			standard error		.06		13
	difference required to be significant				.17		38
	130 to 210 lbs.						
5	12	209	53	130	1.59	4.63	282
12	17	187	49	131	1.84	4.52	245
10	22	193	55	128	1.56	4.47	302
			standard error		.07		11
	difference required to be significant				.20		32
	40 to 210 lbs.						
9	12	77	166	40	.88	2.79	332
12	17	70	166	38	1.09	2.76	252
11	22	67	166	41	.98	2.75	282
			standard error		.04		16
	difference required to be significant				.13		48
Experiment 4 — 33 to 70 lbs.							
10	17	78	54	33	.75	1.85	271
10	22	78	51	32	.74	1.94	270
10	27	75	49	34	.78	1.98	254
			standard error		.12		10
	difference required to be significant				.37		31

From the results obtained with pigs of lighter weight, 80 to 125 pounds, 40 to 74 pounds and 33 to 70 pounds, no single level of protein intake could be designated as being the most satisfactory for body gain but the 12% level was definitely unsatisfactory with respect to feed requirement. Woodman and associates ('36) found that "extra protein" was of no value in improving the rate of growth in the period from weaning to 90 pounds, a period in which the value of such protein might be expected to be manifested. It is possible that other factors, some of which may be unrecognized, may influence the growth of pigs at this stage of their growth, more than it is influenced by the protein level, providing that the protein is adequate from the viewpoint of quality. The results of these experiments do not indicate any deficiency in the quality of the protein or any of the rations with the possible exception of the 10% protein ration used in experiment 1.

The results obtained with the lighter pigs are not surprising in view of the difficulties often encountered with pigs of this weight. The general nutritional requirements of such pigs are not very well established and for this reason many workers select pigs for growth studies when they weigh 70 to 80 pounds rather than at weaning time. In the interim between weaning and being placed on experiment, these larger pigs will have had access to feeds which do not impose on them the restrictions of an experimental diet and thus may be able to build up reserves of dietary essentials which may have been depleted during the suckling period. The less thrifty individuals are eliminated during this period and the surviving pigs which attain a weight of 70 to 80 pounds and which are then placed on the experimental regime, can usually be depended on to give a more satisfactory growth response. The findings of Chick and associates ('38 a, b, c) and Hughes ('38, '39) with respect to the requirements of pigs for members of the vitamin B complex illustrate the paucity of knowledge relative to the requirements of swine for specific dietary factors.

Woodman and associates ('36) have conducted individual feeding tests in a manner similar to that of these experiments. In addition these investigators conducted group feeding tests with the same feeds. From their data they have concluded that the group feeding technique, while it lends confirmation to the individual tests, may be of very limited value, due to its lack of sensitiveness, especially when the expected differences to be investigated are small.

It may be argued that differences too small to be detected by the group feeding method may be too small to have any practical significance; nevertheless, the individual feeding method has certain outstanding advantages. The quantity of feed consumed by each pig is known as is its effect on the individual, and at least an attempt can be made to correlate differences in individual performance with food consumption. Such differences may be brought out by the group feeding method, but they may be so obscured by the variability of the individual response among the various group members, that definite assignment of them to feed is impossible. Thus the group feeding method is to be regarded as a link between practical feeding and scientific experimentation and as a means of verification under practical conditions of the individual feeding trial.

The present experiments yield some interesting data on the quantities of feed required per 100 pounds of body increase. In these experiments the average figure for this value is of the order of 285 pounds, the range being from 447 pounds on the 10% protein level (experiment 1) to 229 pounds on the 17% protein level (experiment 3) at body weights from 70 to 156 pounds. These figures give some idea of the efficiency with which feed is converted into body tissue and, on the whole, are much lower than those usually encountered in either group feeding work or under practical feeding conditions. While no definite average figure is available for actual practical conditions with efficient feeding, approximately 400 pounds of concentrates are usually required to produce 100 pounds of body gain. Less than this quantity of feed may be required in many cases but feed requirements below 300 pounds are unusual in general feeding practice.

The system of feeding used in these experiments has consistently given results for feed efficiency of the order herein reported and at the same time the rate of growth has been maintained satisfactorily. It is recognized that in the effort to maintain an equalized feed intake between three pigs, the feed intake of two members of the triplet was limited by that of the third pig, and that in a sense, limited feeding was being practiced on these two pigs. Under such conditions it is apparent that the efficiency of food conversion might have been almost at a maximum, there being no waste and no feed consumed merely in satisfaction of appetite.

Woodman and associates ('36) observed greater thriftiness among pigs fed individually as compared to those fed in groups, the former making the greater gains in the least number of days. These authors suggest that there is a considerable saving of energy when pigs are individually fed as compared to group-fed pigs which by nature use up considerable energy in consequence of "scrambling" at feeding time at the common trough. The logic of their deductions is recognized and this point together with the possibility of a limited feed intake may explain the high efficiency of food conversion obtained in these experiments.

It should be further pointed out, however, that the average daily gains in experiment 2 exceeded those of experiment 3 in practically all instances, a result which may be due to starting the pigs on experiment 3 at a lighter weight (40 pounds) rather than at 80 pounds as in experiment 2. The resulting initial growth of the pigs on experiment 2 was slow due possibly either to an unrecognized deficiency of the ration or to limited initial reserves of some of the pigs.

SUMMARY

The optimum quantity of protein in the ration for pigs for the growth span from 40 to 210 pounds was found to be from 15 to 17%. Levels of protein of 20, 22, 25 and 27% were not outstanding in their superiority to the 15 and 17% levels, but the trend of the results favored the higher levels of protein intake for pigs from weaning up to a weight of approximately 75 pounds. The lower levels of protein intake were superior to the higher levels for pigs between the weights of 120 and 200 pounds. Rations containing 10 and 12% of protein were unsatisfactory.

LITERATURE CITED

CARROLL, W. E., H. H. MITCHELL AND T. S. HAMILTON 1931–1932 Annual Report Illinois Agr. Exp. Sta., pp. 86–87.

CARROLL, W. E., W. P. GARRIGUS, H. H. MITCHELL AND T. S. HAMILTON 1933–1934 Annual Report Illinois Agr. Exp. Sta., pp. 91–93.

CARROLL, W. E., AND H. H. MITCHELL 1934–1935 Annual Report Illinois Agr. Exp. Sta., pp. 105–106.

CARROLL, W. E., W. P. GARRIGUS, H. H. MITCHELL, T. S. HAMILTON AND W. T. HAINES 1935–1936 Annual Report Illinois Agr. Exp. Sta., pp. 93–96.

CARROLL, W. E., AND E. WISE BURROUGHS 1939 An attempt to locate the most advantageous percentage of protein in the ration of growing-fattening pigs of different weights. Proc. Am. Soc. Animal Prod., pp. 132–136.

CHICK, HARRIETTE, T. F. MACRAE, J. P. M. ARCHER AND C. J. MARTIN 1938 a Curative action of nicotinic acid on pigs suffering from the effects of a diet consisting largely of maize. Biochem. J., vol. 32, pp. 10–12.

——— 1938 b Experiments with pigs on a pellagra-producing diet. II. Biochem. J., vol. 32, pp. 844–854.

——— 1938 c The water soluble B-vitamins other than aneurin (vitamin B_1), riboflavin and nicotinic acid required by the pig. Biochem. J., vol. 32, pp. 2207–2224.

HUGHES, E. H. 1938 The vitamin-B complex as related to growth and metabolism in the pig. Hilgardia, vol. 11, pp. 595–612.

——— 1939 The role of riboflavin and other factors of the vitamin-B complex in the nutrition of the pig. J. Nutrition, vol. 17, pp. 527–533.

MITCHELL, H. H., T. S. HAMILTON, W. T. HAINES, W. E. CARROLL AND E. W. BURROUGHS 1936–1937 Annual Report Illinois Agr. Exp. Sta., pp. 115–116.

WOODMAN, H. E., R. E. EVANS, E. H. CALLOW AND M. A. WISHART 1936 The influence of high levels of protein intake on growth conformation and quality in the bacon pig. J. Agr. Sci., vol. 26, pp. 546–619.

BULLETIN 407 APRIL 1941

Levels of Supplementary Protein for Pigs on Pasture

ALFALFA FORAGE

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

Digest

Results of this study indicate that between weaning and 130 pounds, pigs that are grazing on alfalfa or clover pastures should be fed a concentrate mixture containing at least 15 per cent protein.

A concentrate mixture containing 18 per cent protein is recommended for pigs from weaning to 130 pounds while grazing on rape pastures.

Concentrate mixtures containing 12 per cent protein may be fed to pigs from weaning to 70 pounds on alfalfa and clover pasture but will not provide optimum growth and economy of gain. However, after a pig has reached 70 pounds in liveweight, a 12 per cent protein concentrate mixture is sufficient on these forages.

Nine per cent protein in the concentrate mixture is insufficient and mixtures containing more protein should be fed to growing pigs on forage.

A concentrate mixture with a protein content between 15 and 18 per cent should be fed to pigs from weaning to 70 pounds while grazing on forages, as compared to a mixture containing between 17 and 22 per cent for pigs of these weights in the dry lot. See PA. AGR. EXPT. STA. BULLETIN 401.

Levels of Supplementary Protein * for Pigs on Pasture

T. B. KEITH, R. C. MILLER AND M. A. MCCARTY†

THE quantity of protein required in a concentrate mixture for pigs while on pasture depends upon (1) the plant used as a forage and (2) the age of the pig.

A study was made during a period of three years, to determine the optimum quantity of protein required in a concentrate mixture for normal body gain of growing pigs while pasturing on alfalfa, on Dwarf Essex rape, and on red clover. The concentrate mixture was composed of yellow corn, soybean oilmeal, and low protein tankage.

An outline of the study includes: (1) Feeding trials in which a 12 per cent protein concentrate mixture was compared with a 15 per cent protein concentrate mixture, and a 15 per cent protein mixture was compared with an 18 per cent protein mixture, the pigs on all of these rations being pastured on alfalfa. The experimental subjects were fed as matched pairs in individual stalls, each member of each pair receiving daily the same quantity of concentrate. (2) Further comparisons of 9, 12, 15, and 18 per cent protein mixtures when fed to pigs pasturing on Dwarf Essex rape. Each of these pigs was fed its concentrate mixture in an individual stall, and they were fed as quadruplicates, each member of each quadruplicate receiving daily the same quantity of the concentrate. (3) Similar comparisons of the 9, 12, 15, and 18 per cent protein concentrate mixtures when fed to pigs pasturing on red clover. These pigs were fed by the same method used in the rape forage study.

The data of each experiment were summarized to study the gains and feed requirements for the weight intervals between initial and about 70 pounds, between approximately 70 pounds and final weights, and between the initial and final weights.

Table 1.—Average chemical composition of forages.

CROP	DRY MATTER	CRUDE PROTEIN	FAT	CRUDE FIBER	ASH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Alfalfa	34.63	6.97	1.44	8.42	3.14
Dwarf Essex Rape	14.71	3.58	.64	1.33	1.36
Red Clover	48.76	8.44	1.96	12.54	3.30

---

*Authorized for publication March 14, 1941.

†Assistant Professor of Animal Husbandry, Assistant Professor of Agricultural and Biological Chemistry, and Associate Professor of Animal Husbandry, respectively.

Samples of the forages were taken at random from the pasture plots periodically during the progress of each experiment and analyzed for dry matter, crude protein, ether extract (fat), crude fiber, calcium, phosphorus and total ash, the averages of these analyses being given in table 1. Similar analyses of the components of the concentrate mixtures are to be found in table 2.

Table 2.—Average chemical composition of components of concentrate mixtures.

Feed	Dry Matter	Crude Protein	Fat	Crude Fiber	Nitrogen Free Extract	Ash	Calcium	Phosphorus
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Yellow Corn	88.6	8.9	3.1	1.4	74.0	1.2	.05	.34
Tankage	91.8	33.6	10.0		7.0	41.2	14.86	7.05
Soybean Oilmeal	92.1	40.6	5.4	5.7	35.0	5.4	.30	.71

ALFALFA

This experiment was conducted between the dates of July 23 and October 15, 1937, a period of 84 days. Forty purebred pigs of the Duroc Jersey, Poland China, and Chester White breeds were selected for 20 matched pairs. Ten pairs of these pigs were used to study the comparative values of a 12 and a 15 per cent protein concentrate mixture. One member of each pair was fed the 12 and 1 member the 15 per cent protein concentrate mixture. The other ten pairs were used to determine the comparative values of a 15 and an 18 per cent protein concentrate mixture. One member of each pair was fed the 15 and 1 member the 18 per cent protein concentrate mixture. They were fed twice daily in individual stalls, the quantity alloted to each member of each pair being based on the concentrate mixture consumption of that pair member that ate the least.

The constituents of the concentrate mixtures were corn, soybean oilmeal, 34 per cent protein tankage, and salt, table 3.

Table 3.—Composition of concentrate mixtures fed pigs on alfalfa forage.

Protein Content	Corn	Tankage	Soybean Oilmeal	Salt
per cent	*pounds*	*pounds*	*pounds*	*pounds*
12	87.5	9	3	.5
15	76.5	15	8	.5
18	68.5	11	20	.5

The data for the pigs fed alfalfa forage were used for a study of the most efficient concentrate mixture, with respect to protein content, for the growing period between the initial weight and the weekly weight at which each pig surpassed 130 pounds, table 4. In the comparison of the 12 and the 15 per cent protein concentrates, the pigs fed the 15 per cent protein mixture gained on the average .03 pound more per day and required 7 pounds, or 3 per cent, less concentrate for each 100 pounds of gain than the pigs fed the 12 per cent mixture. These differences are not considered to be significant. Of the total of 10 pairs of pigs, in 7 pairs the pig receiving the 15 per cent protein concentrate made the most economical gain, and in 3 pairs the pig receiving the 12 per cent protein concentrate mixture made the most economical gain. The average gains and the concentrate mixture required for each 100 pounds of gain for the pigs fed the 15 and the 18 per cent protein mixtures between the weights of 54 and 131 pounds were approximately the same.

Table 4.—Average age, weight, gain, concentrate consumption, and economy of gain of the pigs fed the 12, 15, and 18 per cent protein concentrate mixtures with alfalfa forage, between the weights of 54 and 130 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Test Period	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
12	10	111	56	56	130	1.34	3.23	241
15	10	111	54	57	130	1.37	3.20	234
15	10	116	57	54	131	1.35	3.11	230
18	9	108	57	55	130	1.34	3.13	234

Results obtained with pigs weighing 70 pounds and carried to a weight of 130 pounds are given in table 5. The data relating to gain and to feed consumption of the pigs of these weights were obtained by using the values for each individual for the weekly weight at which the pig first attained 70 pounds and the weekly weight at which the pig first surpassed 125 pounds.

Table 5.—Average age, weight, gain, concentrate consumption and economy of gain of the pigs fed the 12, 15, and 18 per cent protein concentrate mixtures with alfalfa forage, between the weights of 69 and 131 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Test Period	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
12	10	124	43	71	130	1.36	3.55	261
15	10	124	41	71	130	1.43	3.56	248
15	10	128	43	70	131	1.41	3.45	245
18	9	119	45	69	130	1.40	3.47	248

The pigs fed the 15 per cent protein concentrate mixture required less feed for each 100 pounds of gain while grazing on alfalfa

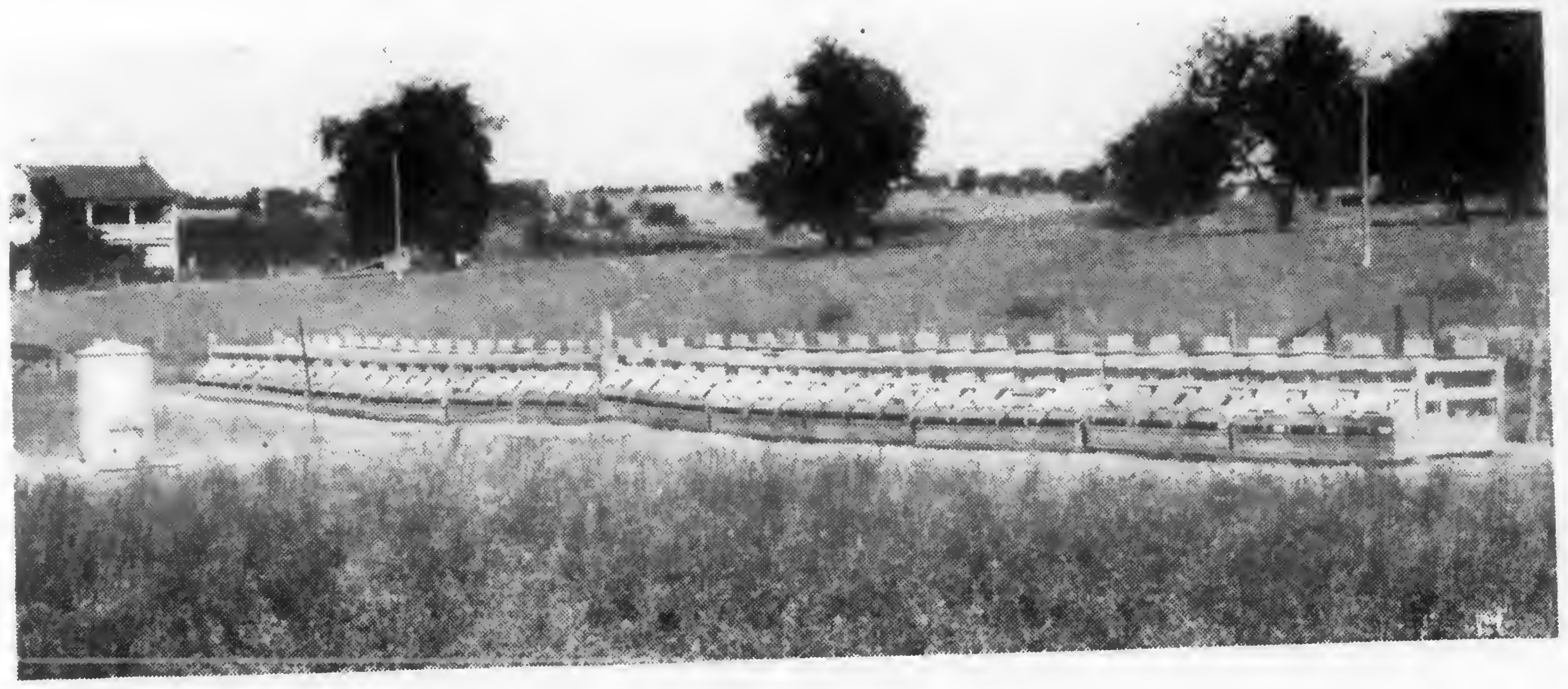


Fig. 1.—Equipment and alfalfa forage in pig pasture.

forage between the weights of 69 and 131 pounds than the pigs fed the 12 or 18 per cent protein concentrate mixtures, table 5. The average daily gain for the pigs fed the 15 per cent protein concentrate mixture was .07 pound or 5 per cent more, and these pigs required 13 pounds or 5 per cent less concentrate mixture for each 100 pounds gain, than the pigs fed the 12 per cent protein concentrate mixture. However, these differences are not considered significant.

The pigs fed the 15 and the pigs fed the 18 per cent protein concentrate mixture made similar average daily gains and required approximately the same quantity of feed for each 100 pounds of gain, table 5.

A study was made of the most efficient content of protein in the concentrate mixture for pigs between weights of 130 and 180 pounds, table 6. The data relating to gain and to concentrate consumption for the pigs of these weights were obtained by using values for each individual for the weekly weight at which the pig first attained 125 pounds, and the final weight of each pig. In the comparison of the 12 and the 15 per cent protein concentrate mixtures, the pigs fed the 15 per cent mixture made the most econom-

Table 6.—Average age, weight, gain, concentrate consumption and economy of gain of the pigs fed the 12, 15, and 18 per cent protein concentrate mixtures with alfalfa forage, between the weights of 130 and 180 pounds.

PROTEIN CONTENT OF CONCENTRATE MIXTURES	NUMBER OF PIGS	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	DAILY GAIN	DAILY RATION	CONCENTRATE REQUIRED PER 100 LB. GAIN
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
12	10	167	28	130	177	1.66	4.87	294
15	10	165	30	130	180	1.67	4.79	287
15	10	176	27	131	175	1.62	4.83	298
18	9	168	27	130	177	1.72	4.75	276

cal gains. The pigs fed the 12 per cent mixture required 7 pounds more concentrate per 100 pounds gain than the pigs fed the 15 per cent mixture. Of the 10 pairs of pigs, in 5 pairs the pig receiving the 15 per cent protein mixture made the most economical gain and in 5 pairs the pig receiving the 12 per cent protein mixture made the most economical gain.

In the comparison of the 15 and the 18 per cent protein concentrate mixtures, the pigs fed the 18 per cent mixture made .10 pound or 6 per cent greater average daily gain and required an average of 22 pounds or 8 per cent, less concentrate for each 100 pounds of gain. Of a total of 9 pairs of pigs, in 5 pairs the pig receiving the 18 per cent protein concentrate mixture made the most economical gain, and in 4 pairs the pig fed the 15 per cent mixture made the cheaper gain. One pig fed the 18 per cent protein concentrate mixture died and the data were not included in this study.

The average daily gain and the average quantity of the concentrate mixture required for each 100 pounds of gain between the weights of 54 and 180 pounds were approximately the same for the pigs fed the 12 and for the pigs fed the 15 per cent protein concentrate, table 7. Of the total of 10 pairs of pigs, in 5 pairs the pig receiving the 12 per cent protein concentrate gained more rapidly, and in 5 pairs the pig receiving the 15 per cent protein concentrate made the greater gain.

Table 7.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 12, 15, and 18 per cent protein concentrate mixtures with alfalfa forage, between the weights of 54 and 180 pounds, a period of 84 days.

PROTEIN CONTENT OF CONCENTRATE MIXTURES	NUMBER OF PIGS	AGE	INITIAL WEIGHT	FINAL WEIGHT	DAILY GAIN	DAILY RATION	CONCENTRATE REQUIRED PER 100 LB. GAIN
per cent		*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
12	10	111	56	177	1.44	3.76	262
15	10	111	57	180	1.47	3.76	257
15	10	116	54	175	1.45	3.68	254
18	10	108	55	171	1.39	3.66	263

In the comparison of the 15 and 18 per cent protein concentrates, the pigs fed the 15 per cent protein mixture gained on the average .06 pound or 4 per cent more per day and required on the average 9 pounds or 3 per cent less concentrate for each 100 pounds of gain than the pigs fed the 18 per cent protein concentrate. Of the total of 10 pairs of pigs, in 6 pairs the pig receiving the 15 per cent protein concentrate gained more rapidly, and in 4 pairs the pig receiving the 18 per cent protein concentrate made the greater gain. This difference is not considered to be statistically significant, table 7.

DWARF ESSEX RAPE

This experiment was conducted between June 14 and September 7, 1938, a period of 85 days. Forty purebred pigs of the Chester White and Berkshire breeds were selected in 10 quadruplicates of the same sex and breed and of approximately the same weight. Two acres of Dwarf Essex rape were used to pasture the 40 pigs.

As soon as the rape was pastured to the extent of there being an insufficient quantity for maximum consumption by all of the experimental pigs, the experiment was terminated.

The quantity of the concentrate mixture alloted to each individual of each quadruplicate was the same, but varied among the quadruplicates. The quantity of the daily mixture alloted to each individual of each quadruplicate was determined by the food consumption of that quadruplicate member which consumed the least concentrate.

The constituents of the concentrate mixtures were the same as those used in the alfalfa experiment, table 8, with the addition of .5 part of ground limestone to the 9 per cent, all corn, concentrate.

Table 8.—Composition of concentrate mixtures fed to pigs on rape forage.

PROTEIN CONTENT OF CONCENTRATE MIXTURES	CORN	TANKAGE	SOYBEAN OILMEAL	SALT	GROUND LIMESTONE
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	99.0			.5	.5
12	87.5	9	3	.5	
15	76.5	15	8	.5	
18	68.5	11	20	.5	

The data for the pigs fed 9, 12, 15, and 18 per cent protein mixtures while pasturing on Dwarf Essex rape were calculated to give information on the most efficient protein concentrate mixture for pigs between the weights of 36 and 73 pounds, table 9, and between the weights of 70 and 129 pounds, table 10. Between the weights of 36 and 70 pounds, the pigs fed the 18 per cent protein concentrate mixture gained on the average 14, 13, and 12 per cent more per day and required 36, 31, and 15 per cent less feed than the pigs fed the 9, 12, and 15 per cent protein mixtures, respectively. The pigs fed the 18 per cent protein mixture required 10 days less time to reach a weight of 72 pounds than the pigs fed the 12 and 9 per cent protein mixtures.

Table 9.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with rape forage, between the weights of 36 and 73 pounds.

PROTEIN CONTENT OF CONCENTRATE MIXTURES	NUMBER OF PIGS	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	DAILY GAIN	DAILY RATION	CONCENTRATE REQUIRED PER 100 LB. GAIN
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	9	74	52	36	72	.72	1.84	255
12	9	73	52	37	73	.73	1.80	247
15	9	76	44	36	68	.74	1.60	217
18	9	77	42	37	72	.84	1.58	188

Fig. 2.—Pigs on Dwarf Essex rape forage.

Between the weights of 70 and 129 pounds, the pigs fed the 15 per cent protein concentrate mixture required less concentrate for each 100 pounds of gain than any of the other groups, table 10. The pigs fed the 15 per cent protein concentrate mixture made 100 pounds gain on 27 pounds less feed than the pigs fed the 12 per cent mixture, and 51 pounds less feed than the pigs fed the 9 per cent mixture. The difference in the quantity of feed required for 100 pounds of gain by the pigs fed the 15 per cent and the pigs fed the 18 per cent protein concentrate mixture was not large enough to be significant. The pigs fed the 12 per cent protein concentrate mixture required 24 pounds less for 100 pounds of gain than the pigs fed the 9 per cent mixture. The concentrate mixture of corn without

Table 10.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with rape forage, between the weights of 70 and 129 pounds.

PROTEIN CONTENT OF CONCENTRATE MIXTURES	NUMBER OF PIGS	AGE	TEST PERIOD	INITIAL WEIGHT	FINAL WEIGHT	DAILY GAIN	DAILY RATION	CONCENTRATE REQUIRED PER 100 LB. GAIN
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	6	122	38	73	114	1.08	3.09	286
12	8	124	35	73	116	1.25	3.28	262
15	8	116	45	70	129	1.32	3.10	235
18	9	119	43	72	125	1.31	3.13	239

a protein supplement did not contain sufficient protein for growing pigs on rape forage.

The pigs fed the 18 per cent protein mixture made more rapid and more economical gains than the pigs fed either the 9, 12, or 15 per cent protein concentrate mixtures, table 11. These pigs made .26, .18, and .06 pound more average daily gain than the pigs fed the 9, 12, and 15 per cent protein mixtures, respectively. They required 83, 28, and 14 pounds, or 27, 11, and 6 per cent less concentrate for 100 pounds of gain, than the pigs fed the 9, 12, and 15 per cent protein mixtures, respectively.

Table 11.—Average age, weight, gain, concentrate consumption, and economy of gains of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with rape forage for a period of 85 days, and between weights of 35 and 120 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	10	74	36	99	.74	2.28	308
12	9	73	37	113	.92	2.33	253
15	10	76	35	116	.94	2.25	239
18	10	77	35	120	1.00	2.25	225

The pigs fed the 15 per cent protein mixture made .02 and .20 pound more average daily gain than the pigs fed the 12 and 9 per cent protein mixtures, respectively. These pigs required 14 and 69 pounds, or 6 and 22 per cent, less concentrate to make 100 pounds of gain than the pigs fed the 12 and 9 per cent mixtures, respectively. One pig fed the 12 per cent protein concentrate mixture died during the first week of the experiment, and the data for this pig were not used.

The pigs fed the 9 per cent protein mixture made the slowest and most expensive gains. These pigs required 55 pounds or 18 per cent more concentrate for each 100 pounds of gain than the pigs fed the 12 per cent protein mixture. The pigs fed the 12 per cent protein mixture made .08 pound greater average daily gain than the pigs fed the 9 per cent mixture.

Of a total of 10 pairs of pigs used in the comparison of the 18 and the 15 per cent protein mixtures, in 5 pairs the pig receiving the 18 per cent protein mixture gained more rapidly, and in 5 pairs the pig receiving the 15 per cent protein mixture made the greater gain.

In the comparison of the 15 and the 12 per cent protein concentrate mixtures, with a total of 9 pairs of pigs, in 8 pairs the pig receiving the 15 per cent protein mixture gained more rapidly and in 1 pair the pig fed the 12 per cent protein mixture made the greater gain. In the comparison of the 9 and 12 per cent protein concentrate mixtures, with a total of 9 pairs of pigs, in 6 pairs the pig receiving the 12 per cent protein mixture gained more rapidly and in 3 pairs the pig fed the 9 per cent protein mixture made the greater gain.

RED CLOVER

This experiment was conducted between June 6 and August 22, 1940, a period of 77 days. Forty purebred pigs of the Chester White and Duroc Jersey breeds were selected to comprise 10 quadruplicates of the same sex and breed and of approximately the same weight. Two acres of red clover divided into 1-acre lots, each for 20 pigs, were used as pasture continuously until depleted. As soon as there was an insufficient quantity of pasture for maximum consumption by all of the experimental pigs, the experiment was terminated.

The method of feeding was the same as that used in the experiment on Dwarf Essex rape. The constituents of the concentrate mixtures were corn, 34 per cent protein tankage, soybean oilmeal, and salt. One-half pound of bonemeal was included in the mixture of corn without supplement. Tankage and soybean oilmeal were used in approximately equal parts, in each of the 12, 15, and 18 per cent protein concentrate mixtures, table 12.

Table 12.—Composition of concentrate mixtures fed pigs on red clover forage.

Protein Content of Concentrate Mixtures	Corn	Tankage	Soybean Oilmeal	Salt	Bone Meal
per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	99.0			.5	.5
12	87.5	6	6	.5	
15	79.5	10	10	.5	
18	68.5	15	16	.5	

Results obtained with pigs weighing 40 pounds and carried to a weight of 73 pounds are given in table 13. The data relating to gain and to concentrate consumption of the pigs of these weights were obtained by using values for each individual from the initial weight and from the weekly weight at which the pig first surpassed 70 pounds.

The pigs between the weights of 37 and 73 pounds fed the 15 per cent protein mixture made more economical gains than the pigs fed the 9 and 12 per cent concentrate mixtures and required 18 and 19 pounds, or 7 and 8 per cent, respectively, less concentrate mixture for each 100 pounds of gain. The pigs fed the 18 per cent pro-

Table 13.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with red clover forage, between the weights of 37 and 73 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Test Period	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	9	112	40	40	72	.82	2.02	248
12	7	113	39	42	73	.86	2.14	249
15	9	115	37	42	71	.83	1.91	230
18	9	114	38	44	73	.81	1.92	237

tein mixture made .02 pound less gain and required 7 pounds more concentrate for each 100 pounds of gain, than the pigs fed the 15 per cent protein mixture. This difference was not significant. The differences in the rate of gain, and the quantity of concentrate ration required for each 100 pounds of gain, for the pigs fed the 12 and the pigs fed the 9 per cent protein rations, were too small to be considered significant.

The data relating to gain and to concentrate consumption of the pigs between the weights of 70 and 137 pounds were obtained by using values for each individual for the weekly weight at which the pig first attained 70 pounds, and the weekly weight at the end of the feeding trial, table 14.

Table 14.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with red clover forage, between the weights of 70 and 137 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Test Period	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	8	132	42	71	122	1.24	4.26	344
12	8	149	43	72	137	1.60	4.28	268
15	10	134	43	70	129	1.38	4.19	304
18	10	149	43	72	129	1.34	4.03	301

The 12 per cent protein concentrate mixture was the most economical for pigs between the weights of 70 and 137 pounds. These pigs made average daily gains .36, .22, and .26 pounds greater, or 29, 16, and 19 per cent more, than the gains of the pigs fed the 9, 15, and 18 per cent protein concentrate mixtures, respectively. The pigs fed the 12 per cent protein concentrate mixture made 100 pounds of gain on 76, 36, and 33 pounds, or 28, 12, and 12 per cent, less concentrate than the pigs fed the 9, 15, and 18 per cent protein concentrate mixtures, respectively.

The 15 per cent protein concentrate mixture was the most economical for the pigs fed red clover forage between the weights of 41 and 137 pounds, table 15. These pigs made an average daily gain of 1.11 pounds and 100 pounds of gain with 286 pounds of concentrate. The average daily gain of 1.11 pounds was .10, .03, and .02 pound greater than that for the pigs fed the 9, 12, and 18 per cent protein concentrate mixtures, respectively. The quantity of concentrate required for each 100 pounds of gain by the pigs fed the 15 per cent protein mixture was 23, 18, and 7 pounds less than that of the pigs fed the 9, 12, and 18 per cent protein concentrate mixtures, respectively. The pigs fed the 9 per cent protein mixture on red clover forage made gains that were not considered sub-normal, yet they required more concentrate for each 100 pounds of gain than the pigs fed the 12, 15, and 18 per cent protein mixtures.

Table 15.—Average age, weight, gain, concentrate consumption, and economy of gain of pigs fed the 9, 12, 15, and 18 per cent protein concentrate mixtures with clover forage for a period of 77 days, between the weights of 41 and 137 pounds.

Protein Content of Concentrate Mixtures	Number of Pigs	Age	Initial Weight	Final Weight	Daily Gain	Daily Ration	Concentrate Required per 100 lb. Gain
per cent		*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
9	9	112	41	119	1.01	3.12	309
12	8	113	45	137	1.08	3.28	304
15	10	115	43	129	1.11	3.18	286
18	10	114	45	129	1.09	3.19	293

SUMMARY

One-hundred-and-twenty pigs were used in a series of feeding trials to study rates of gain and quantities of concentrate mixtures

Fig. 3.—Pigs on red clover forage.

required for each 100 pounds of gain for various weight intervals of pigs fed concentrate mixtures containing 9, 12, 15, and 18 per cent protein while grazing on alfalfa, on Dwarf Essex rape, and on red clover pastures.

The 18 per cent protein concentrate mixture (corn 68.5 parts, tankage 11 parts, soybean oilmeal 20 parts, and salt .5 part) produced the most economical gain for pigs between the weights of 36 and 73 pounds while grazing on Dwarf Essex rape.

The 15 per cent protein concentrate mixture (corn 79.5 parts, tankage 10 parts, soybean oilmeal 10 parts, and salt .5 part) produced the most economical gain for pigs between the weights of 37 and 73 pounds while grazing on red clover pasture.

The 15 per cent protein concentrate mixture produced the most economical gains for pigs between the weights of 70 and 131 pounds while grazing on alfalfa or on Dwarf Essex rape.

The 12 per cent protein concentrate mixture (corn 87.5 parts, tankage 6 parts, soybean oilmeal 6 parts, and salt .5 part) produced the most economical gains for pigs between the weights of 70 and 137 pounds while grazing on red clover.

The 9 per cent protein concentrate mixture (composed of corn 99 parts, salt .5 part, and ground limestone .5 part or bonemeal .5 part) produced normal gains which, however, were not the most economical for pigs of any weight studied in these experiments, while grazing on Dwarf Essex rape or red clover.

Lot I, 1938-39: These steers had corn silage in their fattening ration.

BULLETIN 410 APRIL 1941

ALFALFA-MOLASSES SILAGE AS A ROUGHAGE FOR FATTENING STEERS

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

Lot II, 1938-39: These steers had alfalfa-molasses silage in their ration.

ALFALFA-MOLASSES SILAGE

As a Roughage for Fattening Steers[*]

P. T. Ziegler, F. L. Bentley and R. C. Miller[†]

CLIMATIC conditions along the eastern seaboard during the month of June are not favorable for the making of a good grade of alfalfa hay. Three or four consecutive days of low humidity, bright sunshine and good air circulation are rare. The weather chart for the central Pennsylvania district shows an average of but 14 fair days in the month of June over the 10-year period, 1930 to 1940. The average rainfall for June from 1894 to 1940 was 4¼ inches or about a half-inch greater than the average for the months of July, August, or September. These conditions are favorable to good pastures but not to the making of hay at the proper time or in the proper manner.

Making grass into silage is not a new idea. It has been practiced in a limited way for over 50 years. However, grass silages without preservatives are adapted to only very short feeding periods because they develop bad odors and become unpalatable when held until spring.

The preservation of grass and legume silages by the addition of either an acid or some form of carbohydrate has been a development of the past quarter century.

MAKING ALFALFA-MOLASSES SILAGE

Fermentation of silage is caused in great part by bacteria which need fermentable carbohydrates for their development. This fermentation process produces the acid which preserves the silage, and imparts an agreeable acid odor and taste. Corn is rich in carbohydrates and thus makes an excellent silage. Legumes, however, are high in protein and alkaline material and low in carbohydrates and do not furnish the proper medium for the development of acid forming bacteria. It is necessary, therefore, to add preservative to the cut grass in the form of a mineral acid such as phosphoric of high grade or in the form of carbohydrates, for stimulating acid fermentation. Beet or blackstrap molasses, or corn meal, when added to grass as it is blown into a silo, supply the needed carbohydrates. Because it is cheap, non-corrosive, easy to apply, and produces a tasty silage with a good odor, molasses has come to be the most generally used preservative.

---

[*] Authorized for publication April 2, 1941.

[†] Professor of Animal Husbandry; Head, Department of Animal Husbandry; and Assistant Professor of Agricultural and Biological Chemistry, respectively.

The alfalfa used in these experiments was third cutting in the 1937-38 trial and first cutting in the 1938-39 and 1939-40 trials. It was cut in early bloom, and hauled to the silo without much wilting, with the exception of the year 1938-39 when it was left to wilt for three to four hours.

In the 1937-38 trial, the molasses was run into the cutter box by the gravity system, using 50 pounds per ton of green alfalfa. In 1938-39 and 1939-40, the molasses was pumped to the top of the blower pipe, using 50 pounds of molasses per ton in each instance. The alfalfa was cut into half-inch lengths and one man was placed in the silo after it was half full.

The silo was constructed of hollow tile and reinforced with steel rods every 3½ feet. Sinnce silage exerts almost 40 per cent more pressure on the inside of a silo than does corn silage, it is important that the silo be well constructed and in good repair.

Dates silo was filled.—For the 1937-38 feeding season the silo was filled on September 7 and 8, 1937; for the 1938-39 feeding season the silo was filled on June 16 and 17, 1938; and for the 1939-40 feeding season the silo was filled on June 13 and 14, 1939.

OBJECT OF EXPERIMENT

In the autumn of 1937, when this project was inaugurated, very little information was available on the use of grass silage as a roughage for feed lot use. Dairymen were using grass silage in increasing quantity with very satisfactory results. Cattle feeders had begun to wonder whether grass silage might have a place in their feeding and fattening operations and were asking such questions as:

Since alalfa hay is an important roughage in Pennsylvania cattle feeding, would it be possible to use alfalfa in the form of silage and take advantage of added nutritive value saved by eliminating loss of the leaf and leaching loss by rains? Would the use of alalfa molasses silage save the purchasing of protein supplements? If legume and grass silages are rich in carotene, would feeding them to fattening steers produce yellow fat on the carcasses?

To find the answers, this plan was followed:

PLAN OF EXPERIMENT

Roughage.—Alfalfa molasses silage was made as already explained under "Making Alfalfa-Molasses Silage." Alfalfa hay was made at the same time from the same field from which the silage was made. Plenty of good grade corn silage was available.

Grain.—All three lots of steers received the same amount of corn-and-cob meal. Lot I received added protein in the form of pea size 41 per cent soybean oilmeal, expeller process. The corn-and-cob meal was hammer mill ground, using a three-quarter-inch screen.

Rations.—The rations fed the steers were:

LOT I.—Corn-and-cob meal; Soybean oil meal, pea size; Corn silage.

LOT II.—Corn-and-cob meal; Alfalfa-molasses silage.

LOT III.—Corn-and-cob meal; Alfalfa hay.

Cattle and feeding practice.—Each feeding season, 1937-38, 1938-39, and 1939-40 thirty-six head of steers, grading as good, were fed for a period of 168 days. They were divided into three lots of 12 head each, averaging 625 pounds per steer (average for the three trials.) They were fed twice daily, 6:30 a. m. and 4:30 p. m., the silage and hay following the grain. Water and salt were available in each feed lot at all times. Individual weights were taken every 28 days. Initial and final weights were the average of three consecutive days of weighing. Roughage analyses were made every 28 days.

Minerals.—In the 1937-38 experiment, Lot I had available a constant supply of the following mixture: 50 pounds of salt, 25 pounds of ground limestone, and 25 pounds of steamed bone meal. Lots II and III had a constant supply of salt.

In 1938-39, salt and ground limestone were available to Lot I free choice. Salt was before Lots II and III at all times.

In 1939-40, all three lots were furnished salt and ground limestone free choice.

Table 1.—Chemical analyses of roughages fed steers in a 3-year comparative feeding test of corn silage, alfalfa-molasses silage, and alfalfa hay.*

ROUGHAGE	DRY MATTER	CRUDE PROTEIN	CALCIUM
	per cent	*per cent*	*per cent*
1937 corn silage	23.87	1.88	.07
1938 corn silage	26.85	2.19	.08
1939 corn silage	27.28	2.10	.13
1937 alfalfa-molasses silage	23.45	4.84	.29
1938 alfalfa-molasses silage	31.64	5.83	.55
1939 alfalfa-molasses silage	28.89	4.89	.43
1937 alfalfa hay	84.68	15.19	1.28
1938 alfalfa hay	83.69	13.84	1.13
1939 alfalfa hay	86.41	15.23	1.29

*Average of six analyses each year.

The third cutting alfalfa used in 1937-38 was fine-stemmed and leafy and contained about 7 per cent more moisture than the first cutting used in 1938-39 and 1939-40. Considerable seepage from the silo occurred with the third cutting. Excellent quality alfalfa-molasses silage in respect to color, aroma, and taste resulted in all three years. No difficulty was experienced in getting the steers to eat the alfalfa silage. Some scouring was noticed at first but it never became a troublesome factor.

Table 2.—Summary of results of 1937-38 steer-feeding experiment covering 168 days in which corn silage, alfalfa-molasses silage, and alfalfa hay were compared as roughages.

	LOT I	LOT II	LOT III
No. of steers per lot	12	12	12
Av. initial wt. per steer	630 lb.	627 lb.	629 lb.
Av. final wt. per steer	980 lb.	1023 lb.	988 lb.
Av. daily gain per steer	2.08 lb.	2.36 lb.	2.13 lb.
Total gain per steer	350 lb.	396 lb.	359 lb.
Cost of feed per cwt. gain*	$9.00	$8.50	$9.52
Initial cost per cwt.	7.35	7.35	7.35
Initial cost per cwt. in lots	8.70	8.70	8.70
Selling price per cwt.	8.65	8.65	8.65
Net return per steer	—3.55	—1.81	—5.46
Return per cwt. corn-and-cob meal	.84	.92	.76
Feed consumed per head daily:			
Corn-and-cob meal	13.6 lb.	13.6 lb.	13.6 lb.
Corn silage	12.2 lb.		
Alfalfa-molasses silage		21.00 lb.	
Alfalfa hay			8.86 lb.
Soybean oilmeal	1.05 lb.		
Salt	3 oz.	1.3 oz.	1.09 oz.
Ground limestone	1 oz.		
Feed required per cwt. gain:			
Corn-and-cob meal	654 lb.	577 lb.	637 lb.
Corn silage	589 lb.		
Alfalfa-molasses silage		890 lb.	
Alfalfa hay			415 lb.
Soybean oilmeal	51 lb.		

*Feed prices: corn and cob meal, $1 per cwt.; corn silage, $5 per ton; alfalfa-molasses silage, $6 per ton; alfalfa hay, $15 per ton; soybean oilmeal, $33 per ton; molasses, $16.50 per ton or 10 cents per gallon.

In the 1937-38 feeding experiment in which third cutting alfalfa was used in making the silage and hay fed to Lots II and III respectively, the steers fed alfalfa-molasses silage made the greatest and most economical gains. The Lot II steers were not any higher in finish than those of Lot III but were slightly more growthy. On the same grain ration, Lot II on alfalfa-molasses silage consumed about 9 pounds more silage per head per day than was consumed by Lot I which received corn silage. On the basis of dry matter fed in the 3 rations, Lot I produced 1 pound of gain on 7.62 pounds of dry matter; Lot II required, 7.18 pounds; and Lot III, 9.11 pounds.

Table 3.—Dry matter and protein required per pound of gain in 1937-38 steer feeding experiment.

LOT No.	TOTAL GAIN	DRY MATTER TOTAL	DRY MATTER PER LB. GAIN	PROTEIN TOTAL	PROTEIN PER LB. GAIN
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
I	4196	31998	7.62	2983	.71
II	4753	34120	7.18	3702	.78
III	4310	39290	9.11	4363	1.01

The steers were marketed in Altoona, Pa., and dressed weights and carcass grades were secured from the packer. The steers in Lot I had a slightly higher yield in pounds of beef but did not grade as many choice carcasses as did the Lot III cattle. Lot II and III each contained one carcass which had a slightly yellow cast to the fat.

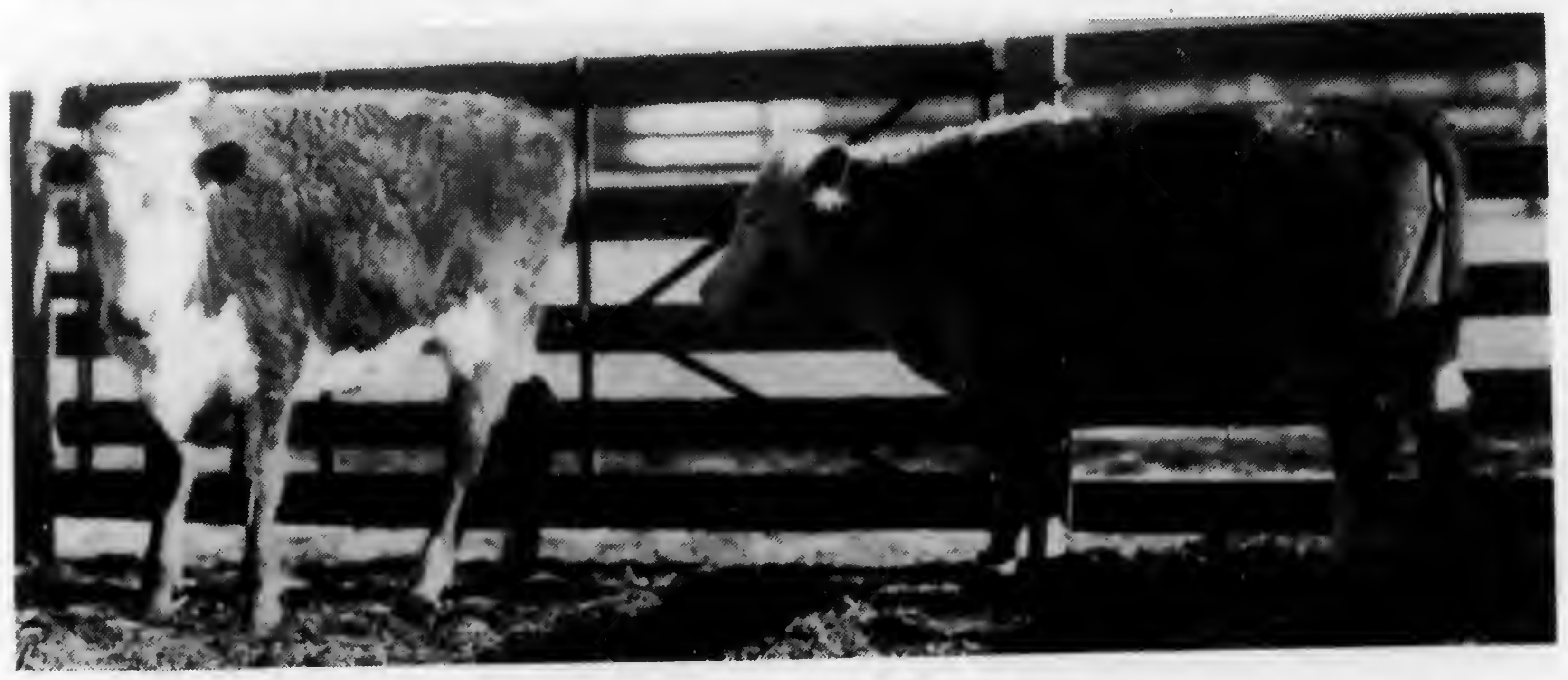
Table 4.—Carcass grades and yields of steers in 1937-38 feeding test.

LOT NO.	CHOICE	GOOD	COMMERCIAL	YIELD (warm wt.)
				per cent
I	5	6	1	59.15
II	4	8		59.3
III	6	6		59.2

Fig. 1.—The above steers are Lot II at the beginning of the 1937-38 feeding experiment. They are typical of the cattle used in all three lots for the three years.

Representative steers from Lot I at beginning of 168-day feeding test.

Representative steers from Lot II at beginning of 168-day feeding test.



Representative steers from Lot III at beginning of 168-day feeding test.

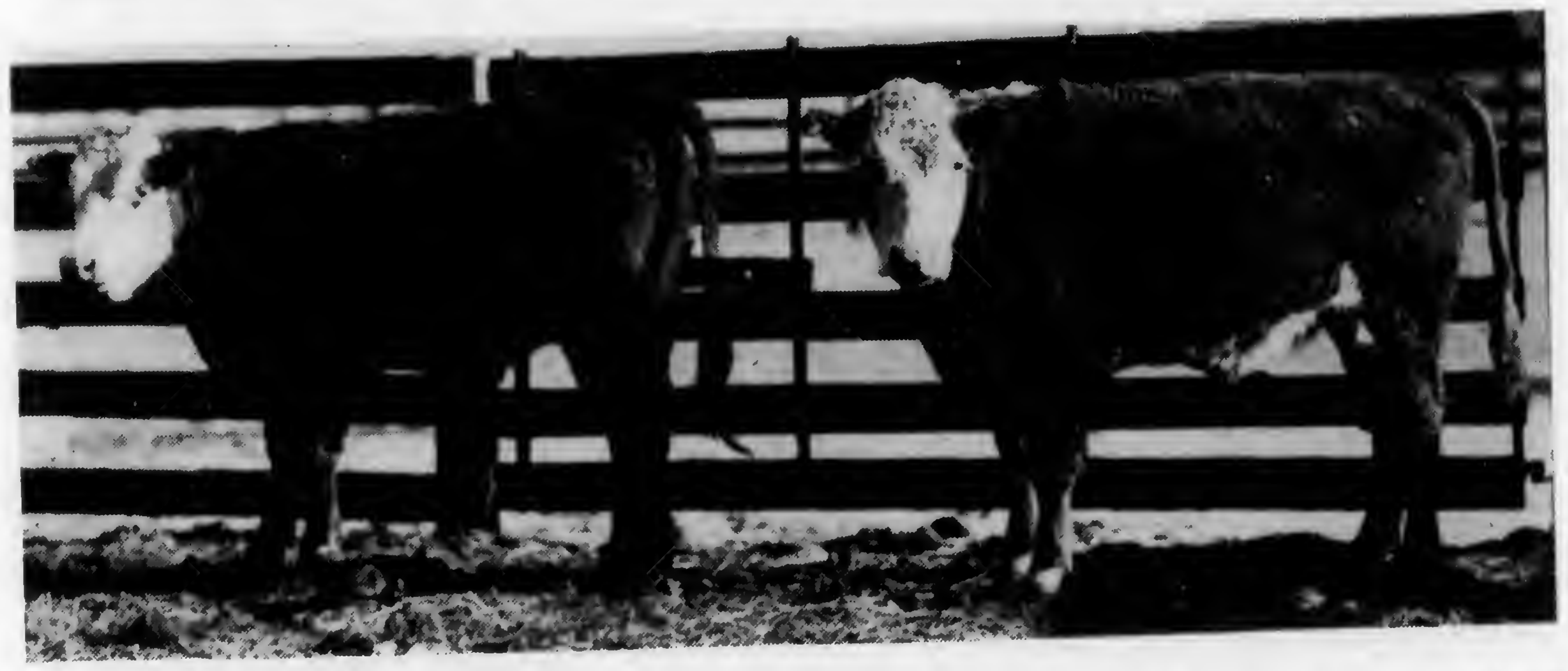
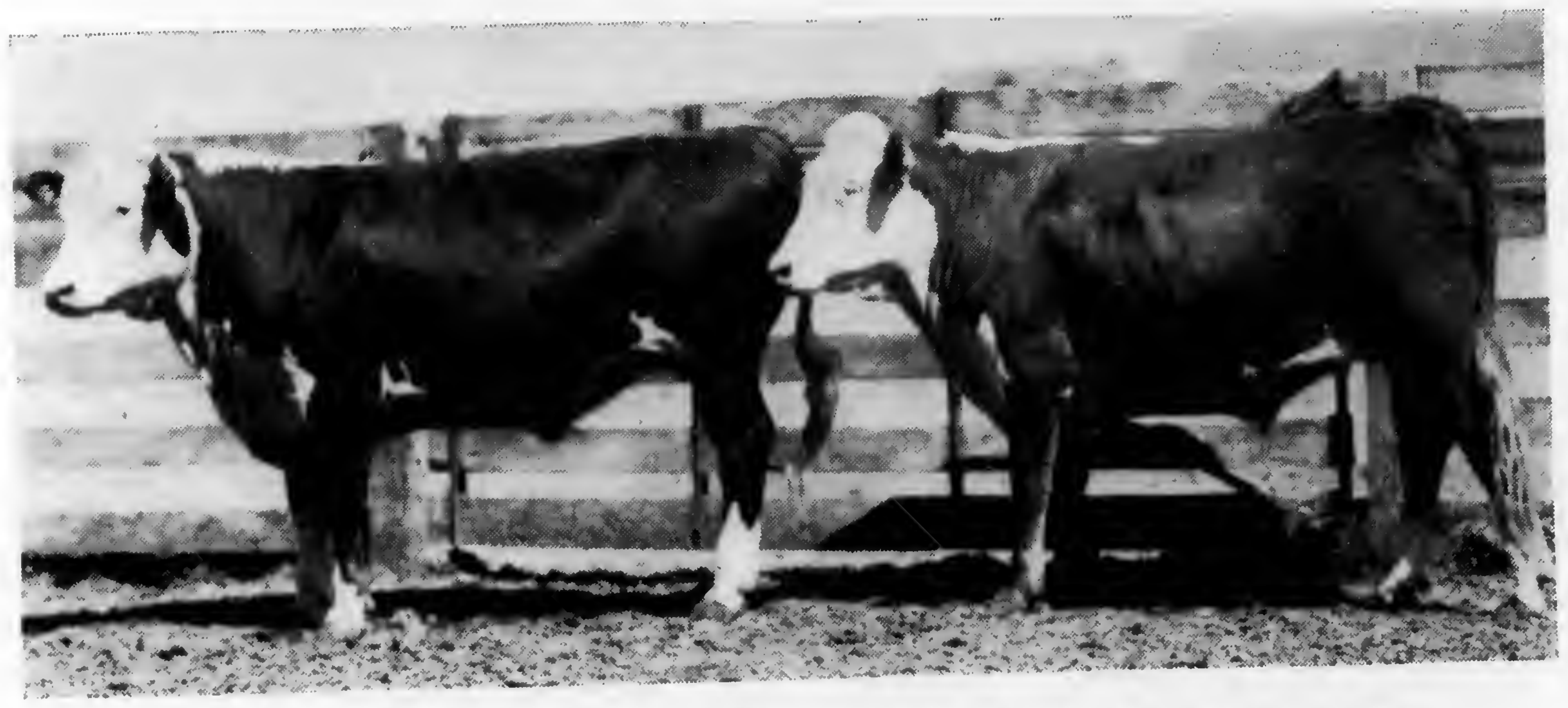


Fig. 2.—Each of these three lots of steers received the same amount of corn-and-cob meal. For roughage, Lot I received corn silage; Lot II, alfalfa-molasses silage; and Lot III, alfalfa hay. Lot I received sufficient soybean oilmeal to bring the protein level up to that of Lots II and III.

Typical steers from Lot I at the end of the feeding test.

Typical steers from Lot II at the end of the feeding test.



Typical steers from Lot III at the end of the feeding test.

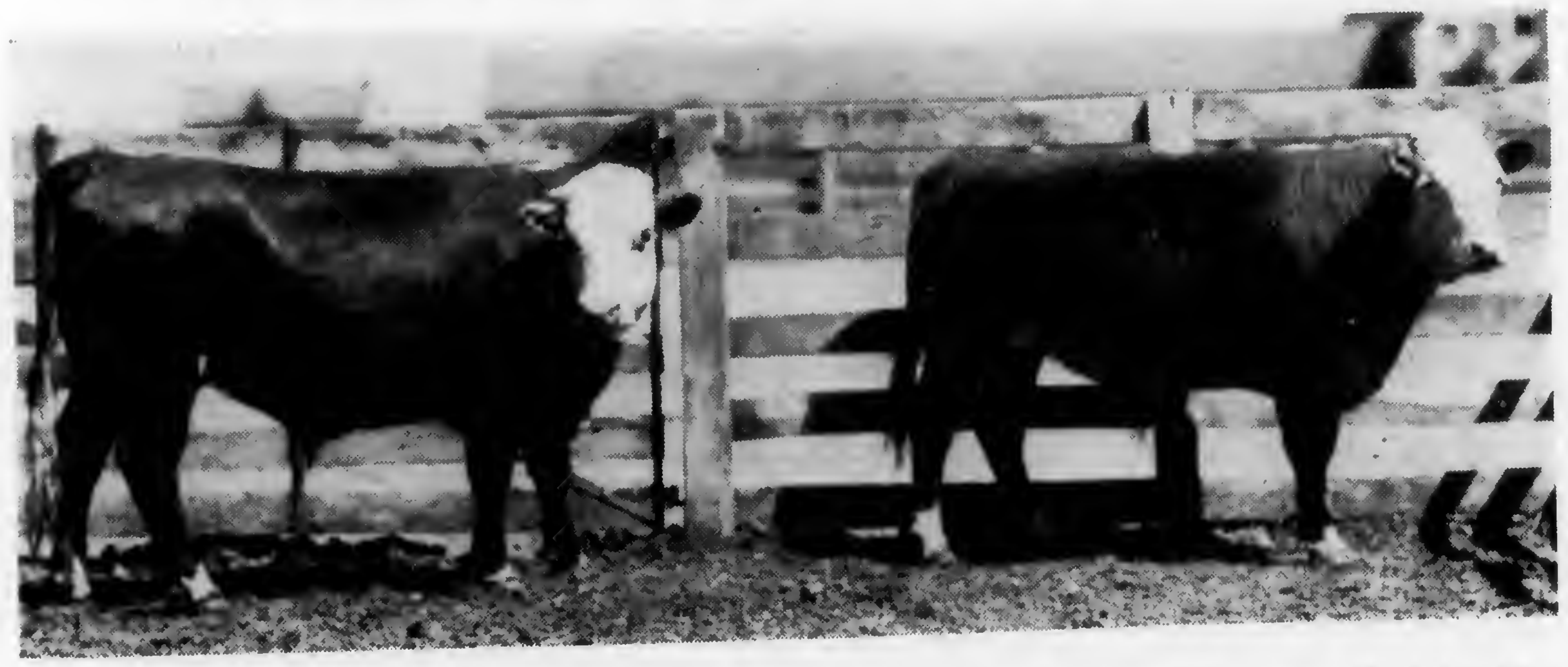


Fig. 3.—These steers as well as those shown in figure 1 were fed in the 1938-39 feeding experiment, and the pairs from each lot are the same animals except in Lot II in which one animal is the same, the other in this picture being a substitute.

Table 5.—Summary of results of 1938-39 steer feeding experiment, 168 days.

	LOT I	LOT II	LOT III
No. of steers per lot	12	12	12
Av. initial wt. per steer	628 lb.	634 lb.	636 lb.
Av. final wt. per steer	978 lb.	960 lb.	963 lb.
Av. daily gain per steer	2.08 lb.	1.94 lb.	1.94 lb.
Total gain per steer	350 lb.	326 lb.	327 lb.
Cost of feed per cwt. gain.*	$8.45	$8.78	$8.86
Initial cost per cwt.	7.75	7.75	7.75
Initial cost per cwt. in lots	9.15	9.15	9.15
Selling price per cwt.	9.20	9.10	9.20
Net return per steer	.18	—1.96	—-1.29
Return per cwt. corn-and-cob meal	.96	.85	.88
Feed consumed per head daily:			
Corn-and-cob meal	11.64 lb.	11.64 lb.	11.64 lb.
Corn silage	13.95 lb.		
Alfalfa-molasses silage		19.74 lb.	
Alfalfa hay			8.15 lb.
Soybean oilmeal (pea size)	1.53 lb.		
Salt	1.5 oz.	1.07 oz.	1.03 oz.
Ground limestone	3.6 oz.		
Feed required per cwt. gain:			
Corn-and-cob meal	560 lb.	599 lb.	599 lb.
Corn silage	671 lb.		
Alfalfa-molasses silage		1016 lb.	
Alfalfa hay			419 lb.
Soybean oilmeal (pea size)	74 lb.		

*Feed prices: corn-and-cob meal, 95 cents per cwt.; corn silage, $5.50 per ton; alfalfa-molasses silage, $6 per ton; alfalfa hay, $15 per ton; soybean oilmeal, $33 per ton; molasses, $16.50 per ton.

The grain level of the 1938-39 experiment was reduced 2 pounds per head daily under that of the previous year to induce the steers to consume more roughage. On the contrary, Lots II and III consumed less roughage which might be expected of Lot II because of the higher dry matter content of the silage, Table 1.

Table 6.—Dry matter and protein required per pound gain in 1938-39 steer feeding experiment.

		DRY MATTER		PROTEIN	
LOT No.	TOTAL GAIN	Total	Per lb. gain	Total	Per lb. gain
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
I	4194	31035	7.40	3296	.79
II	3919	43258	11.04	3729	.95
III	3922	34414	8.77	3682	.94

Table 7.—Carcass grades and yields of steers in 1938-39 feeding test.

LOT No.	CHOICE	GOOD	COMMERCIAL	YIELD (warm wt.)
				per cent
I	6	6		59.8
II	4	7	1	59.0
III	3	8	1	60.1

The 1938-39 experiment showed no significant difference between the three lots either in rate or economy of gain except that the amount of dry matter necessary to produce 1 pound of gain in Lot II was 3½ pounds greater than in Lot I.

The Lot I steers were a very uniform group and carried a smooth covering of white fat. Lots II and III each contained one carcass which ribbed down dark. There were no carcasses carrying yellow fat. Lot III cattle lacked the finish of the same lot in 1937.

Table 8.—Summary of results of 1939-40 steer feeding experiment, 168 days.

	LOT I	LOT II	LOT III
No. of steers per lot	13	13	13
Initial wt. per steer	624 lb.	622 lb.	626 lb.
Final wt. per steer	1023 lb.	984 lb.	984 lb.
Av. daily gain per steer	2.37 lb.	2.15 lb.	2.12 lb.
Total gain per steer	399 lb.	362 lb.	358 lb.
Cost of feed per cwt. gain*	$8.84	$8.59	$8.67
Initial cost per cwt.	8.65	8.65	8.65
Initial cost per cwt. in lots	9.90	9.90	9.90
Selling price per cwt.	9.35	9.35	9.35
Net return per steer	—4.45	—3.29	—3.65
Return per cwt. corn-and-cob meal	.81	.84	.83
Feed consumed per head daily:			
Corn-and-cob meal	12.56 lb.	12.56 lb.	12.56 lb.
Corn silage	19.12 lb.		
Alfalfa-molasses silage		19.59 lb.	
Soybean oilmeal	1.88 lb.		
Alfalfa hay			7.75 lb.
Salt	.9 oz.	1.28 oz.	1.28 oz.
Ground limestone	1.2 oz.	.8 oz.	.8 oz.
Feed required per cwt. gain:			
Corn-and-cob meal	529 lb.	583 lb.	590 lb.
Corn silage	805 lb.		
Alfalfa-molasses silage		908 lb.	
Alfalfa hay			364 lb.
Soybean oilmeal	76 lb.		

*Feed prices: corn-and-cob meal, $1 per cwt.; corn silage, $5 per ton; alfalfa-molasses silage, $6 per ton; soybean oilmeal, $33 per ton; alfalfa hay, $15 per ton; molasses $16.50 per ton.

The third cutting alfalfa used in 1937-38 was fine-stemmed and leafy and contained about 7 per cent more moisture than the first cutting used in 1938-39 and 1939-40. Considerable seepage from the silo occurred with the third cutting. Excellent quality alfalfa-molasses silage in respect to color, aroma, and taste resulted in all three years. No difficulty was experienced in getting the steers to eat the alfalfa silage. Some scouring was noticed at first but it never became a troublesome factor.

Table 2.—Summary of results of 1937-38 steer-feeding experiment covering 168 days in which corn silage, alfalfa-molasses silage, and alfalfa hay were compared as roughages.

	LOT I	LOT II	LOT III
No. of steers per lot	12	12	12
Av. initial wt. per steer	630 lb.	627 lb.	629 lb.
Av. final wt. per steer	980 lb.	1023 lb.	988 lb.
Av. daily gain per steer	2.08 lb.	2.36 lb.	2.13 lb.
Total gain per steer	350 lb.	396 lb.	359 lb.
Cost of feed per cwt. gain*	$9.00	$8.50	$9.52
Initial cost per cwt.	7.35	7.35	7.35
Initial cost per cwt. in lots	8.70	8.70	8.70
Selling price per cwt.	8.65	8.65	8.65
Net return per steer	—3.55	—1.81	—5.46
Return per cwt. corn-and-cob meal	.84	.92	.76
Feed consumed per head daily:			
Corn-and-cob meal	13.6 lb.	13.6 lb.	13.6 lb.
Corn silage	12.2 lb.		
Alfalfa-molasses silage		21.00 lb.	
Alfalfa hay			8.86 lb.
Soybean oilmeal	1.05 lb.		
Salt	3 oz.	1.3 oz.	1.09 oz.
Ground limestone	1 oz.		
Feed required per cwt. gain:			
Corn-and-cob meal	654 lb.	577 lb.	637 lb.
Corn silage	589 lb.		
Alfalfa-molasses silage		890 lb.	
Alfalfa hay			415 lb.
Soybean oilmeal	51 lb.		

*Feed prices: corn and cob meal, $1 per cwt.; corn silage, $5 per ton; alfalfa-molasses silage, $6 per ton; alfalfa hay, $15 per ton; soybean oilmeal, $33 per ton; molasses, $16.50 per ton or 10 cents per gallon.

In the 1937-38 feeding experiment in which third cutting alfalfa was used in making the silage and hay fed to Lots II and III respectively, the steers fed alfalfa-molasses silage made the greatest and most economical gains. The Lot II steers were not any higher in finish than those of Lot III but were slightly more growthy. On the same grain ration, Lot II on alfalfa-molasses silage consumed about 9 pounds more silage per head per day than was consumed by Lot I which received corn silage. On the basis of dry matter fed in the 3 rations, Lot I produced 1 pound of gain on 7.62 pounds of dry matter; Lot II required, 7.18 pounds; and Lot III, 9.11 pounds.

Table 3.—Dry matter and protein required per pound of gain in 1937-38 steer feeding experiment.

		DRY MATTER		PROTEIN	
LOT NO.	TOTAL GAIN	TOTAL	PER LB. GAIN	TOTAL	PER LB. GAIN
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
I	4196	31998	7.62	2983	.71
II	4753	34120	7.18	3702	.78
III	4310	39290	9.11	4363	1.01

The steers were marketed in Altoona, Pa., and dressed weights and carcass grades were secured from the packer. The steers in Lot I had a slightly higher yield in pounds of beef but did not grade as many choice carcasses as did the Lot III cattle. Lot II and III each contained one carcass which had a slightly yellow cast to the fat.

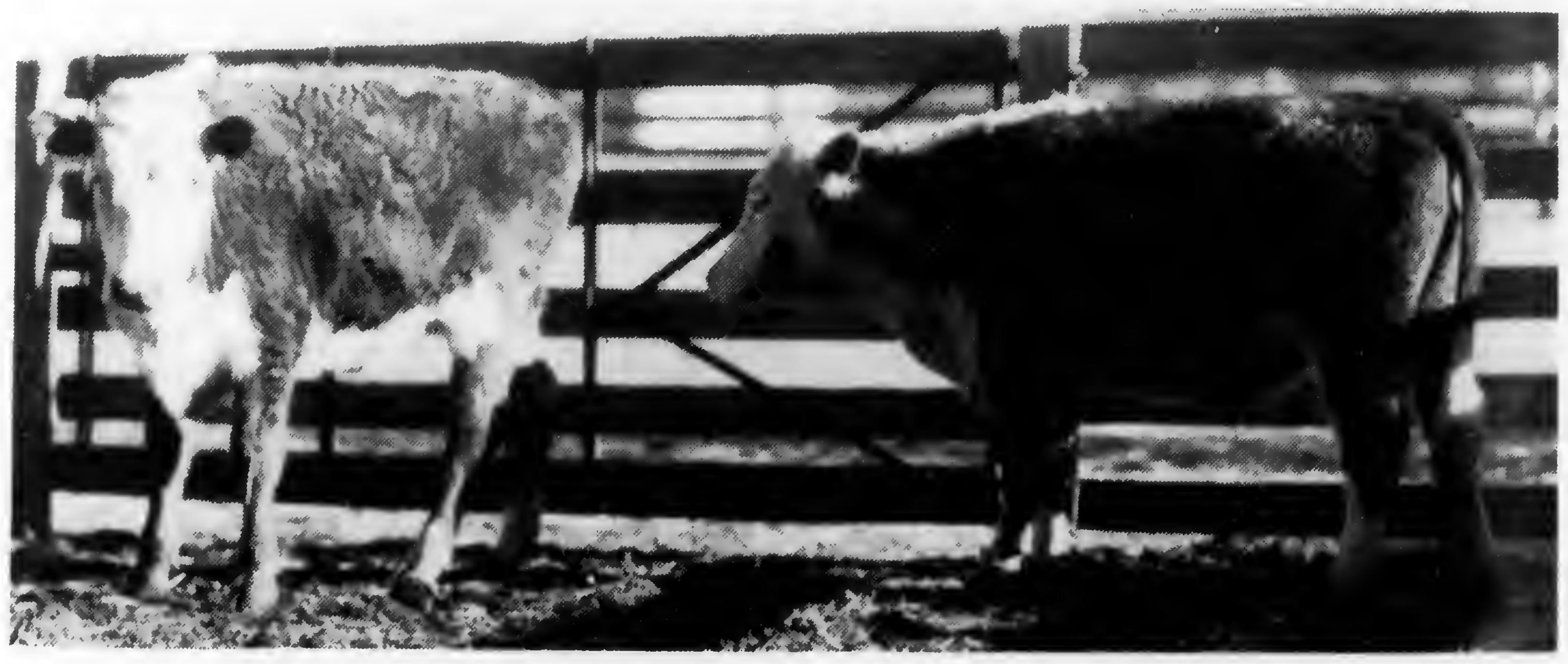
Table 4.—Carcass grades and yields of steers in 1937-38 feeding test.

LOT NO.	CHOICE	GOOD	COMMERCIAL	YIELD (warm wt.)
				per cent
I	5	6	1	59.15
II	4	8		59.3
III	6	6		59.2

Fig. 1.—The above steers are Lot II at the beginning of the 1937-38 feeding experiment. They are typical of the cattle used in all three lots for the three years.

Representative steers from Lot I at beginning of 168-day feeding test.

Representative steers from Lot II at beginning of 168-day feeding test.



Representative steers from Lot III at beginning of 168-day feeding test.

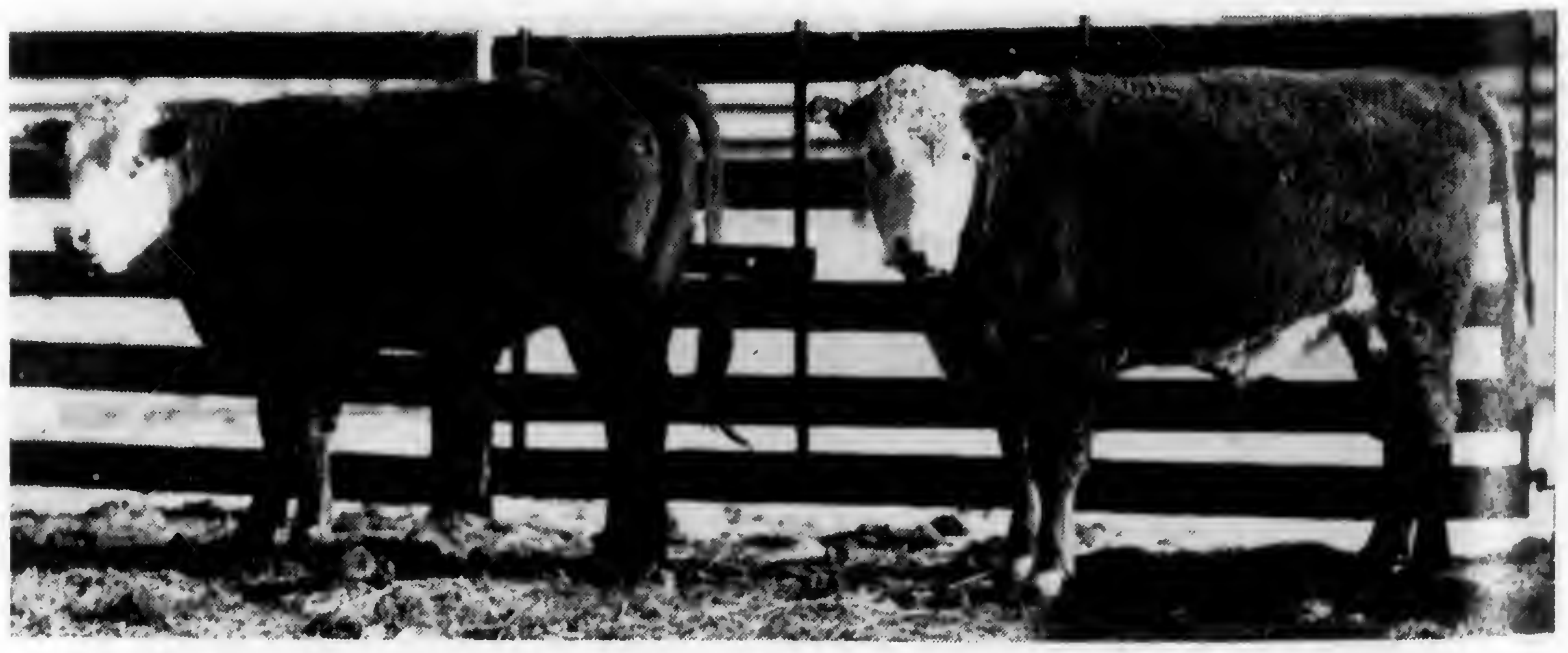
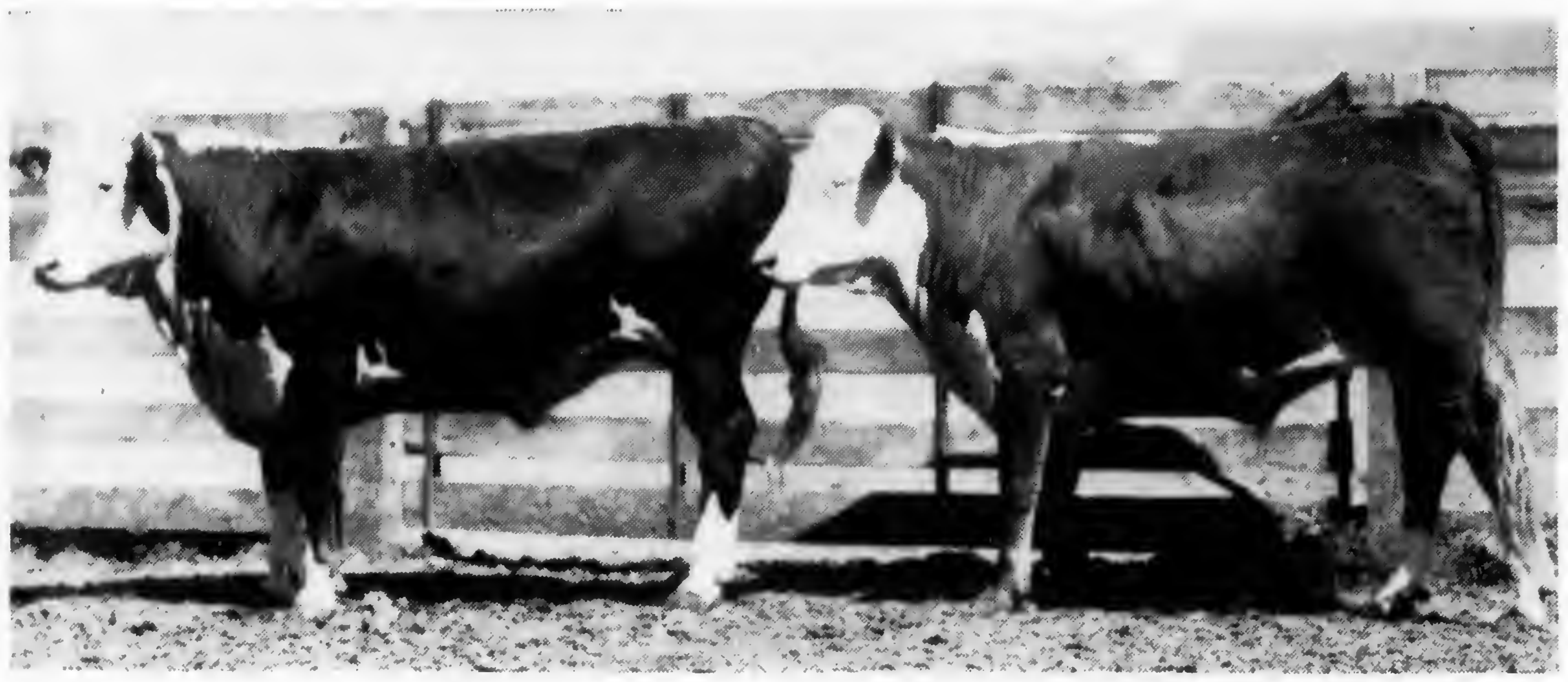


Fig. 2.—Each of these three lots of steers received the same amount of corn-and-cob meal. For roughage, Lot I received corn silage; Lot II, alfalfa-molasses silage; and Lot III, alfalfa hay. Lot I received sufficient soybean oilmeal to bring the protein level up to that of Lots II and III.

Typical steers from Lot I at the end of the feeding test.

Typical steers from Lot II at the end of the feeding test.



Typical steers from Lot III at the end of the feeding test.

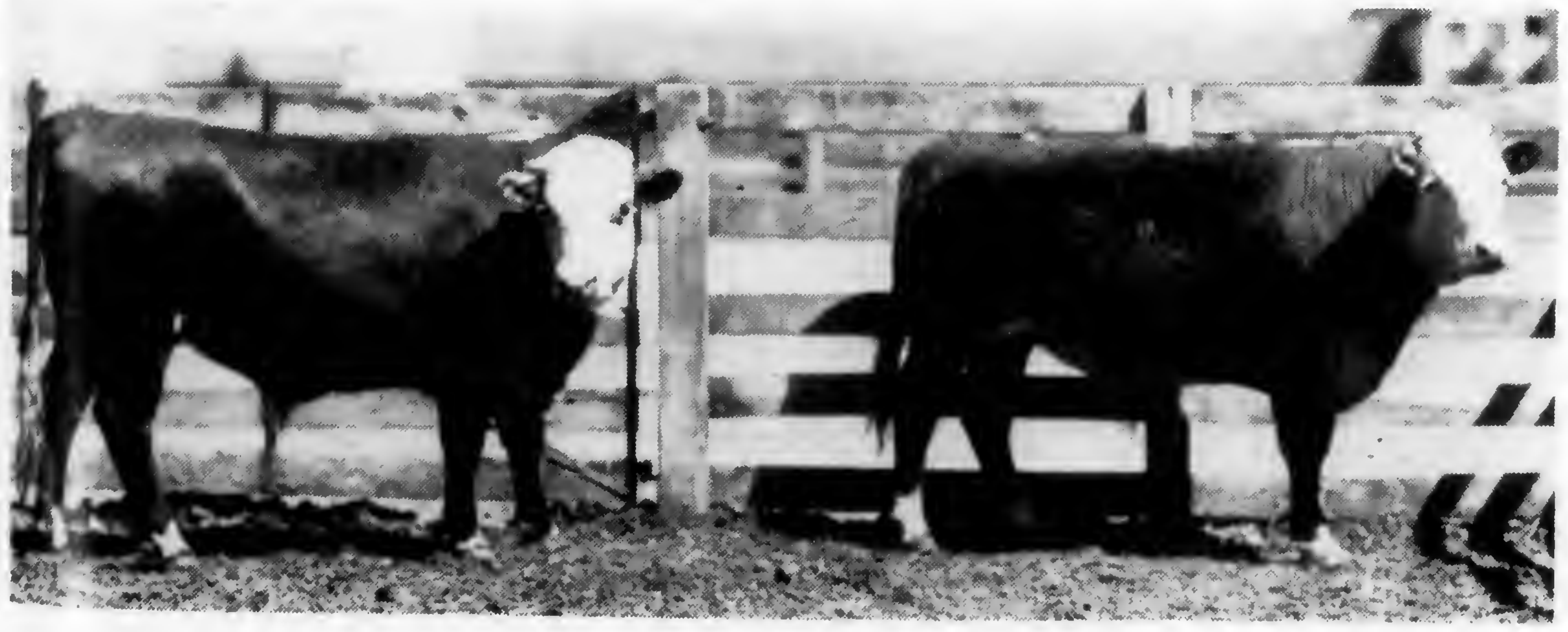


Fig. 3.—These steers as well as those shown in figure 1 were fed in the 1938-39 feeding experiment, and the pairs from each lot are the same animals except in Lot II in which one animal is the same, the other in this picture being a substitute.

Table 5.—Summary of results of 1938-39 steer feeding experiment, 168 days.

	LOT I	LOT II	LOT III
No. of steers per lot	12	12	12
Av. initial wt. per steer	628 lb.	634 lb.	636 lb.
Av. final wt. per steer	978 lb.	960 lb.	963 lb.
Av. daily gain per steer	2.08 lb.	1.94 lb.	1.94 lb.
Total gain per steer	350 lb.	326 lb.	327 lb.
Cost of feed per cwt. gain.*	$8.45	$8.78	$8.86
Initial cost per cwt.	7.75	7.75	7.75
Initial cost per cwt. in lots	9.15	9.15	9.15
Selling price per cwt.	9.20	9.10	9.20
Net return per steer	.18	—1.96	—1.29
Return per cwt. corn-and-cob meal	.96	.85	.88
Feed consumed per head daily:			
Corn-and-cob meal	11.64 lb.	11.64 lb.	11.64 lb.
Corn silage	13.95 lb.		
Alfalfa-molasses silage		19.74 lb.	
Alfalfa hay			8.15 lb.
Soybean oilmeal (pea size)	1.53 lb.		
Salt	1.5 oz.	1.07 oz.	1.03 oz.
Ground limestone	3.6 oz.		
Feed required per cwt. gain:			
Corn-and-cob meal	560 lb.	599 lb.	599 lb.
Corn silage	671 lb.		
Alfalfa-molasses silage		1016 lb.	
Alfalfa hay			419 lb.
Soybean oilmeal (pea size)	74 lb.		

*Feed prices: corn-and-cob meal, 95 cents per cwt.; corn silage, $5.50 per ton; alfalfa-molasses silage, $6 per ton; alfalfa hay, $15 per ton; soybean oilmeal, $33 per ton; molasses, $16.50 per ton.

The grain level of the 1938-39 experiment was reduced 2 pounds per head daily under that of the previous year to induce the steers to consume more roughage. On the contrary, Lots II and III consumed less roughage which might be expected of Lot II because of the higher dry matter content of the silage, Table 1.

Table 6.—Dry matter and protein required per pound gain in 1938-39 steer feeding experiment.

		DRY MATTER		PROTEIN	
LOT No.	TOTAL GAIN	Total	Per lb. gain	Total	Per lb. gain
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
I	4194	31035	7.40	3296	.79
II	3919	43258	11.04	3729	.95
III	3922	34414	8.77	3682	.94

Table 7.—Carcass grades and yields of steers in 1938-39 feeding test.

LOT No.	CHOICE	GOOD	COMMERCIAL	YIELD (warm wt.)
				per cent
I	6	6		59.8
II	4	7	1	59.0
III	3	8	1	60.1

The 1938-39 experiment showed no significant difference between the three lots either in rate or economy of gain except that the amount of dry matter necessary to produce 1 pound of gain in Lot II was 3½ pounds greater than in Lot I.

The Lot I steers were a very uniform group and carried a smooth covering of white fat. Lots II and III each contained one carcass which ribbed down dark. There were no carcasses carrying yellow fat. Lot III cattle lacked the finish of the same lot in 1937.

Table 8.—Summary of results of 1939-40 steer feeding experiment, 168 days.

	LOT I	LOT II	LOT III
No. of steers per lot	13	13	13
Initial wt. per steer	624 lb.	622 lb.	626 lb.
Final wt. per steer	1023 lb.	984 lb.	984 lb.
Av. daily gain per steer	2.37 lb.	2.15 lb.	2.12 lb.
Total gain per steer	399 lb.	362 lb.	358 lb.
Cost of feed per cwt. gain*	$8.84	$8.59	$8.67
Initial cost per cwt.	8.65	8.65	8.65
Initial cost per cwt. in lots	9.90	9.90	9.90
Selling price per cwt.	9.35	9.35	9.35
Net return per steer	—4.45	—3.29	—3.65
Return per cwt. corn-and-cob meal	.81	.84	.83
Feed consumed per head daily:			
Corn-and-cob meal	12.56 lb.	12.56 lb.	12.56 lb.
Corn silage	19.12 lb.		
Alfalfa-molasses silage		19.59 lb.	
Soybean oilmeal	1.88 lb.		
Alfalfa hay			7.75 lb.
Salt	.9 oz.	1.28 oz.	1.28 oz.
Ground limestone	1.2 oz.	.8 oz.	.8 oz.
Feed required per cwt. gain:			
Corn-and-cob meal	529 lb.	583 lb.	590 lb.
Corn silage	805 lb.		
Alfalfa-molasses silage		908 lb.	
Alfalfa hay			364 lb.
Soybean oilmeal	76 lb.		

*Feed prices: corn-and-cob meal, $1 per cwt.; corn silage, $5 per ton; alfalfa-molasses silage, $6 per ton; soybean oilmeal, $33 per ton; alfalfa hay, $15 per ton; molasses $16.50 per ton.

Table 9.—Dry matter and protein required per pound gain in 1939-40 steer feeding experiment.

LOT No.	TOTAL GAIN	DRY MATTER Total	DRY MATTER Per lb. gain	PROTEIN Total	PROTEIN Per lb. gain
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
I	5190	39291	7.57	4214	.81
II	4708	36513	7.76	3743	.80
III	4648	38780	8.34	4226	.88

In the 1939-40 experiment, the grain level was raised about a pound over that of 1938-39, from 11.64 to 12.56 pounds corn-and-cob meal daily. The outstanding difference in food intake between Lot I in the 1939-40 experiment and the same lot in the two previous years was the marked increase in the daily consumption of corn silage. In the two previous trials, the steers were fed hay alone for several weeks to regain the fill lost in shipment. In 1939-40 all three lots were fed all the corn silage they would eat for 10 days to give them fill. This gave the corn silage lot a longer orientation period and they continued to consume a heavy daily feed of silage throughout the entire 168 days.

Although the gains were greater for Lot I, the cost of 100 pounds of gain was also greater and the net return per steer less than in Lots II and III. The purchase of soybean oilmeal at carlot rates at $27 per ton instead of $33 as charged in the experiment makes a difference of 20 cents in the cost per 100 pounds gain. Grass silage

Table 10.—Carcass grades and yields of steers in 1939-40 feeding test.

LOT I No.	LOT I Dr. Wt.	LOT I Grade	LOT I Color Units	LOT II No.	LOT II Dr. Wt.	LOT II Grade	LOT II Color Units	LOT III No.	LOT III Dr. Wt.	LOT III Grade	LOT III Color Units
58	572	good	1.1	53	588	ch.	1.3	51	596	ch.	1.0
52	608	ch.	1.5	54	646	good	3.0*	55	600	good	1.6
56	630	good	1.5	61	487	med.	1.0	57	662	good	1.0
60	512	good	1.5	63	581	good	1.3	59	604	ch.	1.5
66	614	good	1.4	65	556	good	2.3	62	555	ch.	1.0
68	615	good	2.3	72	597	ch.	2.1	64	556	ch.	1.4
71	613	ch.	1.0	76	616	ch.	1.7	67	616	ch.	2.0
73	582	good	2.5	77	606	good	2.5	69	525	good	1.0
74	636	good	2.4	79	602	good	2.3	70	541	good	2.1
82	672	ch.	2.1	80	601	ch.	2.2	75	646	ch.	2.3
83	685	ch.	2.2	85	600	good	1.0	78	580	good	1.[illegible]
86	668	ch.	1.2	87	673	ch.	1.8	81	584	good	1.0
92	595	ch.	1.9	91	607	good	1.2	88	681	good	2.[illegible]

	LOT I	LOT II	LOT III
Average dressed wt.	622 pounds	597 pounds	588 pounds
Average color units	1.74	1.82	1.54
Average yield (warm)	61.9 per cent	61.7 per cent	60 per cent

Table 11.—A 3-year summary of feeding tests of alfalfa-molasses silage as a roughage for fattening steers, 1937-1940.

168-DAY FEEDING PERIOD	WEIGHT PER STEER (start)	WEIGHT PER STEER (end)	AVERAGE DAILY GAIN PER STEER	COST PER CWT. GAIN	COST OF STEERS PER CWT. IN LOTS	SELLING PRICE LESS MARKETING CHARGE	FEED COST PER STEER OTHER THAN CORN-AND-COB MEAL	RETURN PER 100 LB. CORN-AND-COB MEAL	CARCASS GRADE Ch.	CARCASS GRADE G.	CARCASS GRADE M.	DRESSING PER CENT (warm wt.)	SLIGHTLY YELLOW FAT
LOT I	*pounds*	*pounds*	*pounds*										
1937	630	980	2.08	$9.00	$8.70	$8.50	$ 8.60	.84	5	7		59.15	
1938	628	978	2.08	8.45	9.15	9.10	10.91	.96	6	6		59.8	
1940	624	1023	2.37	8.84	9.90	9.25	14.17	.81	6	7		61.9	
Av.	627	994	2.18	8.76	9.25	8.95	11.23	.87	17	20		60.3	
LOT II													
1937	627	1023	2.36	8.50	8.70	8.50	10.81	.92	4	8		59.3	1
1938	634	960	1.94	8.78	9.15	9.00	10.05	.85	4	7	1	59.0	
1939	622	984	2.15	8.59	9.90	9.25	10.01	.84	5	7	1	61.7	1
Av.	627	989	2.15	8.62	9.25	8.92	10.29	.87	13	22	2	60.0	
LOT III													
1937	629	988	2.13	9.52	8.70	8.50	11.30	.76	6	6		59.2	1
1938	636	963	1.94	8.86	9.15	9.10	10.33	.88	3	8	1	60.1	
1939	626	984	2.12	8.67	9.90	9.25	10.73	.83	6	7		60.0	
Av.	630	978	2.06	9.02	9.25	8.95	10.78	.82	15	21	1	59.8	

	LOT I Corn-and-cob meal	LOT I Corn silage	LOT I Soybean oilmeal	LOT II Corn-and-cob meal	LOT II Alfalfa-molasses silage	LOT III Corn-and-cob meal	LOT III Alfalfa hay
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Average daily ration							
1937-38	13.6	12.2	1.05	13.6	21.00	13.6	8.86
1938-39	11.64	13.95	1.53	11.64	19.74	11.64	8.15
1939-40	12.56	19.12	1.88	12.56	19.59	12.56	7.75
Feed consumed per 100 lb. gain							
1937-38	654	589	51	577	890	637	415
1938-39	560	671	74	599	1016	599	419
1939-40	529	805	79	583	908	590	364
Dry matter to produce 100 lb. gain							
1937-40		753		851		873	
Crude protein to produce 100 lb. gain							
1937-40		77		84		95	

plays an important role in furnishing protein in a fattening ration for steers.

There was less difference in the amount of dry matter and crude protein required to make 1 pound of gain in the three lots than in any of the previous experiments. The gains were very similar and cattle as a whole were more uniformly finished than in 1937 and 1938.

In the 1939-40 experiment, a fat sample was taken from the rib of each carcass and the color determined with a Lovibond tintometer. Anything over 2.5 was graded as slightly yellow. Only one carcass which was found in Lot II was slightly yellow. The dressed yield of the 1939 cattle was higher than in the two previous experiments.

SUMMARY

Each of three Lots of good steers during each of three feeding seasons received the same amount of corn-and-cob meal for their grain ration. In addition Lot I received sufficient protein supplement in the form of pea size soybean oilmeal to keep the protein level similar to Lots II and III. The grain ration was limited but roughages were fed according to appetite.

For roughage Lot I received corn silage, Lot II received alfalfa-molasses silage (50 lb. molasses per ton), and Lot III received alfalfa hay made from the same field and cutting as the silage.

The steers originated in Kansas and Texas and averaged 628 pounds in weight. They were marketed after a 168-day feeding period at an average weight of 990 pounds. The bid prices on the three different lots were the same in every instance except Lot II in 1938 which sold 10 cents lower because it was not as highly finished.

The finished steers were marketed in Altoona, which is a 45-mile haul by truck. The average drift on this haul was 1½ per cent. The necessary marketing charge to cover cost of trucking and drift was .15 cents per hundred pounds.

Each year the steers in Lot I carried slightly more finish than the steers of Lot II and III but they were somewhat paunchy in 1939, probably due to the high roughage intake. Lot II cattle were conceded to have tidier middles but appeared a little growthy and not as highly finished, except in 1937 when Lot II steers carried about the same condition as Lot I.

Lot I consumed twice as much ground limestone as either Lot II or III.

No difficulty from scours was experienced in the molasses silage fed group, Lot II.

There was no significant difference between the carcass yields of the three lots but Lot I produced 10 per cent and 5 per cent more choice carcasses respectively than did Lot II and III.

Lot II gave the highest return per hundred-weight of corn-and-cob meal fed.

The cost of 100 pounds of gain over a 3-year period was $8.76 for Lot I; $8.62 for Lot II, and $9.02 for Lot III.

The gains in all three lots were in direct proportion to the amount of corn in the ration.

The 168-day feeding period did not produce any yellow fat on the carcasses.

The results of these three feeding trials would indicate that alfalfa-molasses silage is slightly superior to alfalfa hay of No. 3 grade, made under June weather conditions in Pennsylvania, as a roughage for fattening steers but holds no advantage over corn silage.

The rations of Lots I and II probably should include about a pound of dry roughage per head per day in feedlot practice. It was not included in these trials for experimental reasons.

Reprinted from FEEDSTUFFS *of June 28, 1941*

Recent Developments in Vitamin Research

An Address Delivered at the Convention of the American Feed Manufacturers Association

By DR. R. ADAMS DUTCHER, of Pennsylvania State College

IT must have been about 15 years since I first became familiar with the problems and objectives of the feed manufacturing industry. Since that time, I have had an opportunity, directly and indirectly, to have considerable contact with feed manufacturers and with the technical men associated with the feed industry. In part, my interest has been from a research standpoint; and in part, from the standpoint of training students who go out into the feed manufacturing fields. So I claim some credit for having played a part in the development of the research work that has been done (and some very excellent research work has been and is being done by the industry's laboratories) by furnishing some of the younger men who are now "going places" in the feed research field.

I even claim a little credit for giving Dr. J. E. Hunter an opportunity to develop his scientific talents. My only regret is that I did too good a job. He left me for the industrial field, but my loss was industry's gain. My own research interests, coupled with the fact that I have trained these young men, leads me to hope that I am not going to lose complete touch with the technical workers in the feed industry field.

In many phases of nutrition research, many feed laboratories are far ahead of university laboratories. Many times we swell out our academic chests and point to new discoveries, only to learn that some feed laboratory has worked out these problems months ago.

I am not attempting a scholarly resume of all phases of vitamin research in this paper. I can only point out a few of the recent trends that have been of special interest to me, with the hope that those who have not attempted to follow the voluminous literature may be able to obtain some conception of the general trends as viewed by an academic worker.

SOME GENERAL OBSERVATIONS

Probably the most important development, and certainly the most dramatic, has been the part that modern organic chemistry has played in the major feed nutrition trends of the past few years. Not only has organic chemistry increased the number of vitamins, but vitamins have been isolated in pure form, their structure has been determined, and many of them have been synthesized in the laboratory. In fact, synthetic vitamins are now manufactured in commercial quantities, with the resultant lowering of cost to the consumer as cost of production is lowered and quantity production increased. This not only concerns the purified vitamins, the so-called crystalline products, but also applies to the concentrates that have been developed in com-

In the past few years, a better understanding of the vitamin content of natural food products, and the factors affecting their potency, has been achieved. New and improved vitamin-containing foods are becoming available, such as irradiated products, dehydrated natural products of high potency, special yeast products, riboflavin-rich products produced by special fermentation methods, products of micro-distillation, and concentrates from milk whey, fish liquors and various types of natural products.

While feed and food manufacturers have resorted to fortification or enrichment with vitamins, the policy has not received the blessing of governmental and medical authorities until comparatively recently. The national defense program and lessons learned in Europe have done much to make the public vitamin-conscious.

Last, but not least, alphabetical designations are gradually giving way to such six-dollar words as riboflavin, pantothenic acid, ascorbic acid, pyridoxine, calciferol and activated 7-dehydrocholesterol. These are only the simplest ones. There are between 30 and 35 factors discussed in the run-of-the-mill vitamin literature.

VITAMIN A

The discovery that certain of the carotenoid pigments, specifically beta carotene, serve as pro-vitamins or precursors of vitamin A, is now well known. This does not detract, however, from the dramatic importance of the discovery. Researches of even greater brilliance have shown that light sensitivity and night blindness are usually prevented and cured by ingestion of adequate amounts of carotene and vitamin A. Another outstanding type of research in this field, from a biochemist's viewpoint, was that which is represented by the work of Wald, Hecht and others, leading to the discovery of the mechanism of the photochemical changes in normal vision. They have shown that light decomposes visual purple in the retina of the eye, forming visual yellow (retinene), which, with protein and reserve supplies of vitamin A, again regenerate visual purple. Therefore, the rate of regeneration of visual purple is a rough index of the supply of vitamin A and of the ability of the tissues to utilize it.

The news recently publicized the fact that army physicians have observed that Negroes can see about twice as well in the dark as white soldiers. The possibility of using Negroes as night truck drivers is interesting. This will be a 100% "blackout" so far as a hitch-hiking white rookie is concerned. But seriously, to the biochemist and the student of nutrition, this offers a most intriguing

mother, in order that the newborn will be able to start life without a deficiency of the fat soluble vitamins.

To date, the synthesis of vitamin A has been largely a matter of academic interest. From a practical standpoint, however, Hickman and coworkers, using microdistillation, have succeeded in distilling fat soluble vitamin-containing oils and concentrating such vitamins as A, D and E to an amazingly high degree of potency and purity. Feed manufacturers are already acquainted with this relatively new commercial development.

Evidence has accumulated to prove that two forms of vitamin A exist, namely, vitamin A_1, the one we usually think of in connection with marine fish liver oils; and vitamin A_2, which seems to be characteristic of fresh water fish. The latter differs chemically and physically from the former.

THIAMIN (B_1)

Of all the vitamins, thiamin, or B_1, is the "publicity hound" No. 1. The primary reason for this lies in the fact that it has found wide use in clinical circles, with somewhat dramatic results. Owing to its property of stimulating certain types of biological oxidations, work of the type done at the Mayo clinic has shown that its absence results in lowered vitality and inefficiency. Adequate supplies of thiamin resulted in greater enthusiasm for work and increased stamina. As a result, it has been hailed as the "morale vitamin."

Fundamentally, its clinical and physiological effects go back to the discovery that pyruvic acid, a product of intermediary metabolism, cannot be oxidized unless co-carboxylase is present. Co-carboxylase is a chemical ester of thiamin and pyrophosphoric acid. Without B_1, co-carboxylase cannot be synthesized. When vitamin B_1-depleted rats are injected with thiamin hydrochloride, co-carboxylase is synthesized in the liver and kidney almost immediately.

Medical authorities are reporting almost dramatic results in the use of thiamin in certain types of nervous disorders, and vitamin B_1 is a standard remedy for patients afflicted with alcoholic polyneuritis. British physicians are advocating thiamin for civilians who are undergoing the nerve-shattering ordeal of continuous bombing.

RIBOFLAVIN

Riboflavin is one of the fractions of what was originally called vitamin B_2. It is a yellow fluorescent pigment found in many natural food products, particularly in milk, liver and yeast. It has also been synthesized in the laboratory, and the synthetic product threatens to be a serious competitor of those now made

which is, in reality, a multiple deficiency disease.

NICOTINIC ACID

Nicotinic acid is a simple pyridine carboxylic acid and has been known since the days of Casamir Funk's early work. In fact, he isolated it, but discarded it because it did not cure beri-beri. In other words, it had to be rediscovered 30 years later. Evlehjem and co-workers of Wisconsin are responsible for the discovery that nicotinic acid and its amide are curative in black tongue disease of dogs. This disease can be produced on pellagragenic diets; that is, diets that will produce pellagra in humans. Spies and others have shown that nicotinic acid is primarily responsible for preventing and curing the characteristic bilaterally symmetrical skin lesions of pellagra.

Another fact of considerable physiological interest is that nicotinic acid (in phosphorylated form) also forms co-enzymes which act as hydrogen carriers in bringing about normal oxidation of carbohydrates in living tissue.

ASCORBIC ACID

Ascorbic acid is another pure crystalline product of known chemical structure which is now produced commercially in quantity at low cost. Apparently, it can be synthesized by most species of animals, with the exception of a few species such as man, monkeys and guinea pigs. Since ascorbic acid is readily and reversibly oxidized and reduced, it has created considerable interest as a possible anti-oxidant, thus interesting the food and feed manufacturer. Professor Dahle and his associates at Pennsylvania State College, and others in other laboratories, have shown that they have been able to prevent "off-flavors" in milk by means of additions of ascorbic acid to the milk.

Dr. Macy, in charge of the nutrition work of the Children's Fund of Michigan at Detroit, has a unique set-up for child feeding. She and her co-workers substantiate the findings of Dr. Hess that ascorbic acid and vitamin C-rich foods play an important role in building up resistance to colds. Dr. King, of Pittsburgh, contends that this vitamin is a "decoxicator," building up resistance to toxins and poisons of bacterial origin. He finds that youthful tissues are invariably richer in ascorbic acid than similar tissues of aged people. The mechanism of ascorbic acid synthesis by certain species of animals is still an unsolved problem. Rations high in dextrin seem to cause rats to excrete greater amounts of ascorbic acid, but no clue regarding the synthetic mechanism has been found.

cellent research work has been and is being done by the industry's laboratories) by furnishing some of the younger men who are now "going places" in the feed research field.

I even claim a little credit for giving Dr. J. E. Hunter an opportunity to develop his scientific talents. My only regret is that I did too good a job. He left me for the industrial field, but my loss was industry's gain. My own research interests, coupled with the fact that I have trained these young men, leads me to hope that I am not going to lose complete touch with the technical workers in the feed industry field.

In many phases of nutrition research, many feed laboratories are far ahead of university laboratories. Many times we swell out our academic chests and point to new discoveries, only to learn that some feed laboratory has worked out these problems months ago.

I am not attempting a scholarly resume of all phases of vitamin research in this paper. I can only point out a few of the recent trends that have been of special interest to me, with the hope that those who have not attempted to follow the voluminous literature may be able to obtain some conception of the general trends as viewed by an academic worker.

SOME GENERAL OBSERVATIONS

Probably the most important development, and certainly the most dramatic, has been the part that modern organic chemistry has played in the major feed nutrition trends of the past few years. Not only has organic chemistry increased the number of vitamins, but vitamins have been isolated in pure form, their structure has been determined, and many of them have been synthesized in the laboratory. In fact, synthetic vitamins are now manufactured in commercial quantities, with the resultant lowering of cost to the consumer as cost of production is lowered and quantity production increased. This not only concerns the purified vitamins, the so-called crystalline products, but also applies to the concentrates that have been developed in comparatively recent months.

This has led to more exact experimental work of the biological type. Uncomplicated deficiency symptoms can be produced and cured. This information had to be available before very much progress could be made. Thus has evolved a more complete knowledge of vitamin function. In addition, the existence of pure vitamins has made it possible to create chemical, physical and bacteriological methods of assay, partly replacing tedious, expensive and time-consuming assay methods with experimental animals. Nevertheless, it will be a long time before animal tests can be completely done away with.

The use of pure crystalline vitamins has led to the establishment of international standards for vitamin assay, and to the adoption of the so-called international units of measurement for several of the vitamins. This, in turn, has made it possible to establish minimum vitamin requirements for various species of animals, including man. At least, this problem has been approached with greater confidence.

received the blessing of governmental and medical authorities until comparatively recently. The national defense program and lessons learned in Europe have done much to make the public vitamin-conscious.

Last, but not least, alphabetical designations are gradually giving way to such six-dollar words as riboflavin, pantothenic acid, ascorbic acid, pyridoxine, calciferol and activated 7-dehydrocholesterol. These are only the simplest ones. There are between 30 and 35 factors discussed in the run-of-the-mill vitamin literature.

VITAMIN A

The discovery that certain of the carotenoid pigments, specifically beta carotene, serve as pro-vitamins or precursors of vitamin A, is now well known. This does not detract, however, from the dramatic importance of the discovery. Researches of even greater brilliance have shown that light sensitivity and night blindness are usually prevented and cured by ingestion of adequate amounts of carotene and vitamin A. Another outstanding type of research in this field, from a biochemist's viewpoint, was that which is represented by the work of Wald, Hecht and others, leading to the discovery of the mechanism of the photochemical changes in normal vision. They have shown that light decomposes visual purple in the retina of the eye, forming visual yellow (retinene), which, with protein and reserve supplies of vitamin A, again regenerate visual purple. Therefore, the rate of regeneration of visual purple is a rough index of the supply of vitamin A and of the ability of the tissues to utilize it.

The news recently publicized the fact that army physicians have observed that Negroes can see about twice as well in the dark as white soldiers. The possibility of using Negroes as night truck drivers is interesting. This will be a 100% "blackout" so far as a hitch-hiking white rookie is concerned. But seriously, to the biochemist and the student of nutrition, this offers a most intriguing research problem. Why is it and how is it that the Negro can see better in the dark? Is it a question of his vitamin A storage? Is it a matter of the effect of light on the skin? Is it a pigmentation problem? Certainly, it is a very intriguing problem.

I have been unable to find any evidence that there is much danger of overdosage of vitamin A. Vedder and Rosenberg have fed as much as 100,000 international units of vitamin A daily without ill effects.

Most workers agree that livers of newborn animals and infants are very low in vitamin A. This has been shown to be true for several species. Apparently, the placenta does not permit the passage of fat soluble vitamins to the foetus. If it does, the vitamins must be destroyed after they have been stored. Since foetal blood is lower in vitamin A than the blood of the mother, it would appear that the "placental barrier theory" is probably correct. This points to the necessity of insuring adequate amounts of the fat soluble vitamins, particularly vitamin A, in the diet of the lactating

ly, vitamin A_1, the one we usually think of in connection with marine fish liver oils; and vitamin A_2, which seems to be characteristic of fresh water fish. The latter differs chemically and physically from the former.

THIAMIN (B_1)

Of all the vitamins, thiamin, or B_1, is the "publicity hound" No. 1. The primary reason for this lies in the fact that it has found wide use in clinical circles, with somewhat dramatic results. Owing to its property of stimulating certain types of biological oxidations, work of the type done at the Mayo clinic has shown that its absence results in lowered vitality and inefficiency. Adequate supplies of thiamin resulted in greater enthusiasm for work and increased stamina. As a result, it has been hailed as the "morale vitamin."

Fundamentally, its clinical and physiological effects go back to the discovery that pyruvic acid, a product of intermediary metabolism, cannot be oxidized unless co-carboxylase is present. Co-carboxylase is a chemical ester of thiamin and pyrophosphoric acid. Without B_1, co-carboxylase cannot be synthesized. When vitamin B_1-depleted rats are injected with thiamin hydrochloride, co-carboxylase is synthesized in the liver and kidney almost immediately.

Medical authorities are reporting almost dramatic results in the use of thiamin in certain types of nervous disorders, and vitamin B_1 is a standard remedy for patients afflicted with alcoholic polyneuritis. British physicians are advocating thiamin for civilians who are undergoing the nerve-shattering ordeal of continuous bombing.

RIBOFLAVIN

Riboflavin is one of the fractions of what was originally called vitamin B_2. It is a yellow fluorescent pigment found in many natural food products, particularly in milk, liver and yeast. It has also been synthesized in the laboratory, and the synthetic product threatens to be a serious competitor of those now made by fermentation or by extraction of natural products. Government authorities informed me recently at the Defense Nutrition Conference that sufficient quantities of riboflavin will be available by January, 1942, to require its inclusion in enriched wheat flour, in addition to those supplements that have already been approved.

Riboflavin also becomes phosphorylated to form a co-enzyme for oxidase systems necessary for normal cellular oxidations in the body. Research work in the poultry field has shown that riboflavin is a necessary ingredient of poultry rations and plays an important role in growth and egg production and increases hatchability. Cornell workers have shown that riboflavin is synthesized by micro-organisms in the droppings following defecation, but there is no evidence, they state, that synthesis occurs in the intestinal tract of the bird. This is difficult to understand.

Sebrell and others have proved conclusively that riboflavin is necessary for the prevention and cure of sore mouths (cheilosis), characteristic of pellagra,

and others have shown that nicotinic acid is primarily responsible for preventing and curing the characteristic bilaterally symmetrical skin lesions of pellagra.

Another fact of considerable physiological interest is that nicotinic acid (in phosphorylated form) also forms co-enzymes which act as hydrogen carriers in bringing about normal oxidation of carbohydrates in living tissue.

ASCORBIC ACID

Ascorbic acid is another pure crystalline product of known chemical structure which is now produced commercially in quantity at low cost. Apparently, it can be synthesized by most species of animals, with the exception of a few species such as man, monkeys and guinea pigs. Since ascorbic acid is readily and reversibly oxidized and reduced, it has created considerable interest as a possible anti-oxidant, thus interesting the food and feed manufacturer. Professor Dahle and his associates at Pennsylvania State College, and others in other laboratories, have shown that they have been able to prevent "off-flavors" in milk by means of additions of ascorbic acid to the milk.

Dr. Macy, in charge of the nutrition work of the Children's Fund of Michigan at Detroit, has a unique set-up for child feeding. She and her co-workers substantiate the findings of Dr. Hess that ascorbic acid and vitamin C-rich foods play an important role in building up resistance to colds. Dr. King, of Pittsburgh, contends that this vitamin is a "decoxicator," building up resistance to toxins and poisons of bacterial origin. He finds that youthful tissues are invariably richer in ascorbic acid than similar tissues of aged people. The mechanism of ascorbic acid synthesis by certain species of animals is still an unsolved problem. Rations high in dextrin seem to cause rats to excrete greater amounts of ascorbic acid, but no clue regarding the synthetic mechanism has been found.

ANTI GRAY HAIR FACTOR

While an "anti-bald-headed" vitamin is still being sought for those who would like to grow more hair, it would appear that scientists are definitely getting somewhere in their researches on the "anti-gray hair" vitamin—and many scientific feed workers can tell university people a lot of things regarding this factor, or combination of factors.

Agnes Fay Morgan and other workers have shown that "graying" can be prevented and cured by the so-called filtrate factor obtained from liver and yeast. Fractionation of the extracts has led to the conclusion that a number of factors may be involved. Certainly, pantothenic acid appears to be one of the factors. Beautiful silver gray fur in black (pied) rats can be produced by feeding pantothenic-acid-poor diets, and redeposition of normal black pigment in the hair and skin can be caused by adding pantothenic acid to the diet. There appear to be other factors, however, that affect the efficiency of the pigmentation and depigmentation. Ansbacher has recently ascribed "anti-graying"

REDUCTION
CHANGE
10 X TO 14 X

Reprinted from FEEDSTUFFS *of June 28, 1941*

Recent Developments in Vitamin Research

An Address Delivered at the Convention of the American Feed Manufacturers Association

By DR. R. ADAMS DUTCHER, of Pennsylvania State College

IT must have been about 15 years since I first became familiar with the problems and objectives of the feed manufacturing industry. Since that time, I have had an opportunity, directly and indirectly, to have considerable contact with feed manufacturers and with the technical men associated with the feed industry. In part, my interest has been from a research standpoint; and in part, from the standpoint of training students who go out into the feed manufacturing fields. So I claim some credit for having played a part in the development of the research work that has been done (and some very excellent research work has been and is being done by the industry's laboratories) by furnishing some of the younger men who are now "going places" in the feed research field.

I even claim a little credit for giving Dr. J. E. Hunter an opportunity to develop his scientific talents. My only regret is that I did too good a job. He left me for the industrial field, but my loss was industry's gain. My own research interests, coupled with the fact that I have trained these young men, leads me to hope that I am not going to lose complete touch with the technical workers in the feed industry field.

In many phases of nutrition research, many feed laboratories are far ahead of university laboratories. Many times we swell out our academic chests and point to new discoveries, only to learn that some feed laboratory has worked out these problems months ago.

I am not attempting a scholarly resume of all phases of vitamin research in this paper. I can only point out a few of the recent trends that have been of special interest to me, with the hope that those who have not attempted to follow the voluminous literature may be able to obtain some conception of the general trends as viewed by an academic worker.

SOME GENERAL OBSERVATIONS

Probably the most important development, and certainly the most dramatic, has been the part that modern organic chemistry has played in the major feed nutrition trends of the past few years. Not only has organic chemistry increased the number of vitamins, but vitamins have been isolated in pure form, their structure has been determined, and many of them have been synthesized in the laboratory. In fact, synthetic vitamins are now manufactured in commercial quantities, with the resultant lowering of cost to the consumer as cost of production is lowered and quantity production increased. This not only concerns the purified vitamins, the so-called crystalline products, but also applies to the concentrates that have been developed in comparatively recent months.

This has led to more exact experimental work of the biological type. Uncomplicated deficiency symptoms can be produced and cured. This information had to be available before very much progress could be made. Thus has evolved a more complete knowledge of vitamin function. In addition, the existence of pure vitamins has made it possible to create chemical, physical and bacteriological methods of assay, partly replacing tedious, expensive and time-consuming assay methods with experimental animals. Nevertheless, it will be a long time before animal tests can be completely done away with.

The use of pure crystalline vitamins has led to the establishment of international standards for vitamin assay, and to the adoption of the so-called international units of measurement for several of the vitamins. This, in turn, has made it possible to establish minimum vitamin requirements for various species of animals, including man. At least, this problem has been approached with greater confidence.

In the past few years, a better understanding of the vitamin content of natural food products, and the factors affecting their potency, has been achieved. New and improved vitamin-containing foods are becoming available, such as irradiated products, dehydrated natural products of high potency, special yeast products, riboflavin-rich products produced by special fermentation methods, products of micro-distillation, and concentrates from milk whey, fish liquors and various types of natural products.

While feed and food manufacturers have resorted to fortification or enrichment with vitamins, the policy has not received the blessing of governmental and medical authorities until comparatively recently. The national defense program and lessons learned in Europe have done much to make the public vitamin-conscious.

Last, but not least, alphabetical designations are gradually giving way to such six-dollar words as riboflavin, pantothenic acid, ascorbic acid, pyridoxine, calciferol and activated 7-dehydrocholesterol. These are only the simplest ones. There are between 30 and 35 factors discussed in the run-of-the-mill vitamin literature.

VITAMIN A

The discovery that certain of the carotenoid pigments, specifically beta carotene, serve as pro-vitamins or precursors of vitamin A, is now well known. This does not detract, however, from the dramatic importance of the discovery. Researches of even greater brilliance have shown that light sensitivity and night blindness are usually prevented and cured by ingestion of adequate amounts of carotene and vitamin A. Another outstanding type of research in this field, from a biochemist's viewpoint, was that which is represented by the work of Wald, Hecht and others, leading to the discovery of the mechanism of the photochemical changes in normal vision. They have shown that light decomposes visual purple in the retina of the eye, forming visual yellow (retinene), which, with protein and reserve supplies of vitamin A, again regenerate visual purple. Therefore, the rate of regeneration of visual purple is a rough index of the supply of vitamin A and of the ability of the tissues to utilize it.

The news recently publicized the fact that army physicians have observed that Negroes can see about twice as well in the dark as white soldiers. The possibility of using Negroes as night truck drivers is interesting. This will be a 100% "blackout" so far as a hitch-hiking white rookie is concerned. But seriously, to the biochemist and the student of nutrition, this offers a most intriguing research problem. Why is it and how is it that the Negro can see better in the dark? Is it a question of his vitamin A storage? Is it a matter of the effect of light on the skin? Is it a pigmentation problem? Certainly, it is a very intriguing problem.

I have been unable to find any evidence that there is much danger of overdosage of vitamin A. Vedder and Rosenberg have fed as much as 100,000 international units of vitamin A daily without ill effects.

Most workers agree that livers of newborn animals and infants are very low in vitamin A. This has been shown to be true for several species. Apparently, the placenta does not permit the passage of fat soluble vitamins to the foetus. If it does, the vitamins must be destroyed after they have been stored. Since foetal blood is lower in vitamin A than the blood of the mother, it would appear that the "placental barrier theory" is probably correct. This points to the necessity of insuring adequate amounts of the fat soluble vitamins, particularly vitamin A, in the diet of the lactating mother, in order that the newborn will be able to start life without a deficiency of the fat soluble vitamins.

To date, the synthesis of vitamin A has been largely a matter of academic interest. From a practical standpoint, however, Hickman and coworkers, using microdistillation, have succeeded in distilling fat soluble vitamin-containing oils and concentrating such vitamins as A, D and E to an amazingly high degree of potency and purity. Feed manufacturers are already acquainted with this relatively new commercial development.

Evidence has accumulated to prove that two forms of vitamin A exist, namely, vitamin A_1, the one we usually think of in connection with marine fish liver oils; and vitamin A_2, which seems to be characteristic of fresh water fish. The latter differs chemically and physically from the former.

THIAMIN (B_1)

Of all the vitamins, thiamin, or B_1, is the "publicity hound" No. 1. The primary reason for this lies in the fact that it has found wide use in clinical circles, with somewhat dramatic results. Owing to its property of stimulating certain types of biological oxidations, work of the type done at the Mayo clinic has shown that its absence results in lowered vitality and inefficiency. Adequate supplies of thiamin resulted in greater enthusiasm for work and increased stamina. As a result, it has been hailed as the "morale vitamin."

Fundamentally, its clinical and physiological effects go back to the discovery that pyruvic acid, a product of intermediary metabolism, cannot be oxidized unless co-carboxylase is present. Co-carboxylase is a chemical ester of thiamin and pyrophosphoric acid. Without B_1, co-carboxylase cannot be synthesized. When vitamin B_1-depleted rats are injected with thiamin hydrochloride, co-carboxylase is synthesized in the liver and kidney almost immediately.

Medical authorities are reporting almost dramatic results in the use of thiamin in certain types of nervous disorders, and vitamin B_1 is a standard remedy for patients afflicted with alcoholic polyneuritis. British physicians are advocating thiamin for civilians who are undergoing the nerve-shattering ordeal of continuous bombing.

RIBOFLAVIN

Riboflavin is one of the fractions of what was originally called vitamin B_2. It is a yellow fluorescent pigment found in many natural food products, particularly in milk, liver and yeast. It has also been synthesized in the laboratory, and the synthetic product threatens to be a serious competitor of those now made by fermentation or by extraction of natural products. Government authorities informed me recently at the Defense Nutrition Conference that sufficient quantities of riboflavin will be available by January, 1942, to require its inclusion in enriched wheat flour, in addition to those supplements that have already been approved.

Riboflavin also becomes phosphorylated to form a co-enzyme for oxidase systems necessary for normal cellular oxidations in the body. Research work in the poultry field has shown that riboflavin is a necessary ingredient of poultry rations and plays an important role in growth and egg production and increases hatchability. Cornell workers have shown that riboflavin is synthesized by micro-organisms in the droppings following defecation, but there is no evidence, they state, that synthesis occurs in the intestinal tract of the bird. This is difficult to understand.

Sebrell and others have proved conclusively that riboflavin is necessary for the prevention and cure of sore mouths (cheilosis), characteristic of pellagra, which is, in reality, a multiple deficiency disease.

NICOTINIC ACID

Nicotinic acid is a simple pyridine carboxylic acid and has been known since the days of Casamir Funk's early work. In fact, he isolated it, but discarded it because it did not cure beri-beri. In other words, it had to be rediscovered 30 years later. Evlehjem and co-workers of Wisconsin are responsible for the discovery that nicotinic acid and its amide are curative in black tongue disease of dogs. This disease can be produced on pellagragenic diets; that is, diets that will produce pellagra in humans. Spies and others have shown that nicotinic acid is primarily responsible for preventing and curing the characteristic bilaterally symmetrical skin lesions of pellagra.

Another fact of considerable physiological interest is that nicotinic acid (in phosphorylated form) also forms co-enzymes which act as hydrogen carriers in bringing about normal oxidation of carbohydrates in living tissue.

ASCORBIC ACID

Ascorbic acid is another pure crystalline product of known chemical structure which is now produced commercially in quantity at low cost. Apparently, it can be synthesized by most species of animals, with the exception of a few species such as man, monkeys and guinea pigs. Since ascorbic acid is readily and reversibly oxidized and reduced, it has created considerable interest as a possible anti-oxidant, thus interesting the food and feed manufacturer. Professor Dahle and his associates at Pennsylvania State College, and others in other laboratories, have shown that they have been able to prevent "off-flavors" in milk by means of additions of ascorbic acid to the milk.

Dr. Macy, in charge of the nutrition work of the Children's Fund of Michigan at Detroit, has a unique set-up for child feeding. She and her co-workers substantiate the findings of Dr. Hess that ascorbic acid and vitamin C-rich foods play an important role in building up resistance to colds. Dr. King, of Pittsburgh, contends that this vitamin is a "decoxicator," building up resistance to toxins and poisons of bacterial origin. He finds that youthful tissues are invariably richer in ascorbic acid than similar tissues of aged people. The mechanism of ascorbic acid synthesis by certain species of animals is still an unsolved problem. Rations high in dextrin seem to cause rats to excrete greater amounts of ascorbic acid, but no clue regarding the synthetic mechanism has been found.

ANTI GRAY HAIR FACTOR

While an "anti-bald-headed" vitamin is still being sought for those who would like to grow more hair, it would appear that scientists are definitely getting somewhere in their researches on the "anti-gray hair" vitamin—and many scientific feed workers can tell university people a lot of things regarding this factor, or combination of factors.

Agnes Fay Morgan and other workers have shown that "graying" can be prevented and cured by the so-called filtrate factor obtained from liver and yeast. Fractionation of the extracts has led to the conclusion that a number of factors may be involved. Certainly, pantothenic acid appears to be one of the factors. Beautiful silver gray fur in black (pied) rats can be produced by feeding pantothenic-acid-poor diets, and redeposition of normal black pigment in the hair and skin can be caused by adding pantothenic acid to the diet. There appear to be other factors, however, that affect the efficiency of the pigmentation and depigmentation. Ansbacher has recently ascribed "anti-graying" power to a pure chemical compound, para amino benzoic acid, but his results as yet have not been confirmed by others.

Vitamin B_6, pyridoxine, is now obtainable in crystalline form, and its chemical formula can be written. While it is known as the anti-acrodynia factor, it is not of sufficient practical importance at present to devote much time to it.

OTHER FACTORS

Alpha tocopherol, an alcohol, is also known as vitamin E. It can be manufactured in quantity by microdistillation and seems to function other than as an "anti-sterility" vitamin. Apparently, it plays an important role in preventing and curing certain types of muscular dystrophy and has been described as preventing encephalomalacia in "crazy chicks." Its clinical value is still open to question.

Vitamin D research has shown no pronounced trends in past months other than the tendency to show increased interest in activated 7-dehydrocholesterol as a commercial source of vitamin D for food fortification. With the exception of poultry, activated ergosterol and natural vitamin D seem to compare quite faorably for all practical purposes and can be used more or less interchangeably.

The chemistry of vitamin K, the antihemorrhagic vitamin, has been worked out and a number of related compounds have been found to promote prothrombin formation and hasten blood clotting. Clinical applications continue to appear in the medical literature, and a water soluble form of vitamin K has been described which can now be injected into the blood stream. Apparently, alfalfa leaf meal and putrefying fish continue to be the principal sources of the K vitamins, of which there are two, K_1 and K_2.

Discussion of grass juice factors, gizzard erosion factors, factors U, W and H, lactation factors, etc., will be omitted here because chemists know very little about them.

MISCELLANEOUS PROBLEMS

Since I have devoted considerable time in past years to dairy and livestock problems, I have attempted to follow certain types of researches that are related to these fields.

Dr. Kon and co-workers in England at the National Dairy Research Institute have probably done the most comprehensive work on factors affecting the vitamin content of milk. They have recently restudied the effect of heating and processing of milk. They have described experiments in which milk was flash-heated at 160° F., homogenized, autoclaved at 212° F., the bottles closed and the heating continued to 230° F., and cooled, giving the milk plenty of punishment. They concluded that such treatment did not reduce digestibility, but they felt that the biological value of the proteins had been reduced about 6%. Carotene was not affected (this agrees with our own work) and storage seemed to have no deleterious effect on the A potency. B_1, or thiamin, however, was partly destroyed. Pasteurization destroyed but 10% of the thiamin, while sterilization accounted for about value of raw versus pasteurized milk. Their work with calves and human beings agrees with most other work of similar nature; namely, that no outstanding differences can be noted from a practical standpoint.

The effect of diet on the nutritive value of milk continues to hold the attention of many workers, including ourselves, at Pennsylvania State College. It is now well established that the vitamin A potency of milk is related directly to the carotene content of the ration. Various laboratories over the country have shown that the "pigmented breeds," or Jerseys and Guernseys, can be made to produce practically colorless butterfat if fed for a sufficient length of time on a carotene-free diet. It was concluded many years ago that the so-called pigmented breeds are, in a sense, pathological, going through life with incipient carotinemia, such as human beings occasionally experience. In other words, the Jersey and the Guernsey are less efficient as "carotene converters." The poorer pigmenters, like the Holsteins and Ayrshires, may and often do eat more carotene per day, yet they invariably store and excrete less carotene. Our work shows that the Holstein is a better converter of carotene. As a result, the Holstein stores and excretes a greater amount of the more or less colorless sterol, vitamin A, and less of the orange yellow hydrocarbon pigment, carotene. Gram for gram and pound for pound, butterfats from all these breeds display about the same total biological vitamin A potency when fed to animals. Therefore, butterfats from pigmented breeds are richer in untransformed carotene and poorer in the transformed vitamin A. Conversely, the less pigmented breeds yield butterfats with less carotene but more vitamin A. This means that the unstandardized milks rich in butterfat will contain more vitamin A units or potency per quart than low-fat milks, providing the cows are fed the same ration.

Of all the carotenoid pigments in feeds, the prevailing pigment which passes into the butterfat is beta carotene. If carrots are fed to cows in quantity, alpha carotene is also found in the fat. It is interesting to note that the red pigment, lycopene, found in tomatoes, cannot pass into the butterfat. This red pigment is an isomeric hydrocarbon very similar in structure to carotene. Apparently, the cow has no mechanism whereby she can convey this lycopene to milk. Where the barrier is and how lycopene is selectively excluded, are not known.

Hauge and his co-workers at Purdue state that the cow must receive more than 500,000 units of vitamin A (as carotene) per day in order to produce a butterfat of maximum A potency. These workers have also called attention to the existence of a substance in soybeans which prevents transference of vitamin A from the feed to the milk. They believe the substance is thermostable and that it can be removed by adsorption on Nuchar. It is said to be present in both the bean and the oil. This work will, of course, have to be confirmed before it is definitely accepted.

The question of iodized milks appears in scientific literature from time to time.

complex in the rumen of the dairy cow by micro-organisms. Hunt, with more recent and refined techniques, has found that riboflavin is synthesized in the rumen of the cow in appreciable quantities. This has been our feeling ever since riboflavin was recognized, because our synthetic organism was a yellow pigmenter, which Professor Knutsen named "flavo-bacterium vitarumen."

Hunt doubts whether vitamin B_1 is synthesized in the cow's rumen, while McElroy and Goss of California believe they have proved it to be synthesized in the rumen of sheep. These workers could not prove that it is synthesized in the cow's rumen, although the milk contained normal quantities. A non-fistulated cow did have thiamin in the rumen and they conclude, as we at Pennsylvania did, that B_1 is probably not a dietary essential for ruminants, so far as the feed is concerned. Recently, Hart, Elvehjem and others have advanced evidence to show that thiamin, riboflavin, nicotinic acid, pyridoxine (B_6), pantothenic acid, biotin and vitamin K are all synthesized in the rumen.

Kon of England maintains that sprouted corn caused increased excretion of ascorbic acid (vitamin C) in cows' milk. As early as 1919, our laboratory, as well as those of Hart at Wisconsin and Hess and Supplee in New York, drew similar conclusions regarding the effect of the vitamin C content of the ration on the vitamin C content of the milk. In the intervening years, many laboratories, started by the work of Hughes at Kansas, have questioned the ability of the ration to change the vitamin C content of milk appreciably. Holmes, in a recent paper, finds seasonal fluctuations in the ascorbic acid content of milk, but doubts whether diet or period of lactation have much effect.

We at Pennsylvania have restudied the question recently, using chemical estimations of ascorbic acid, and the old work of 20 years ago is, of course, open to question, since guinea pigs were evidently responding to milk vitamins which at that time had not been discovered. Recently, we restudied the whole situation.

Our first problem, of course, was the collection of milk, blood and urine in order to find out how to estimate the ascorbic acid accurately. We finally satisfied ourselves on the ascorbic acid determination in blood, milk and urine, using the dye titration method, and the Roe furfuraldehyde method.

We used only Holstein cows, to eliminate any variations due to breed, and we fed the largest doses of ascorbic acid or vitamin C that have ever been recorded. We have fed as high as 4,000,000 international units per day, or nearly ½ lb of ascorbic acid in pure crystalline form. Of course, we couldn't inject that much, but we have injected as much as 1,000,000 units per day.

We administered the ascorbic acid to cows in five different ways: (1) by feeding with small amounts of corn silage, (2) by drenching from a bottle, (3) by intravenous injection, (4) by subcutaneous injection, and (5) with gelatin capsules.

We were unable to find an increase in the ascorbic acid content of the blood

tained in the rumen, except that the destruction time was a little longer. Body temperature and aeration through the churning of the rumen contents had much to do with the destruction.

We are now studying the mechanism of this destruction in the rumen. Also, we have evidence that the cow is capable of synthesizing vitamin C.

We are also planning some long-time experiments on vitamin C-free diets, to see just how far we can go in reducing the ascorbic acid content of milk by feeding. We have evidence that we can go as low as 15 milligrams per quart and as high as 30 milligrams per quart by feeding, but we do not yet know if these are the absolute limits.

Whitnah and Ridell at Kansas state that they are able to obtain increases in blood ascorbic acid by feeding fresh rye. We want to repeat their work and compare it with the various sources of ascorbic acid.

We have been interested in the last three or four years in the effect of exercise on vitamin requirement. We have divided our rats into three groups: one group gets no exercise, another group is permitted to exercise voluntarily, and yet another group is forced to exercise by having the rotor run by a motor at the rate of about 1,000 revolutions per day.

Briefly, we found that the fat, lazy rats that got no exercise came down with vitamin A deficiency faster than those that were forced to exercise or which exercised voluntarily. We also found that the exercised rats had better intestinal motility as measured by number and size of fecal pellets.

The work done by Dr. Hunter and co-workers on the vitamin requirements of poultry, as well as by Bechdel and co-workers on the vitamin D requirements of dairy cattle and that of Guilbert and Hart on the vitamin requirements of horses and other forms of livestock, is very important and should be continued. Further data must also be obtained relative to the vitamin requirements of human beings in health and disease.

Twenty years ago, one of my students decided to go into another field of work because he thought that all vitamin problems had been solved. Since that time the whole vitamin field has undergone unbelievable transformations. With all the knowledge we have to date, the field is hardly scratched. Each new discovery opens up hundreds of new problems.

I appeal to the executive officers of the various industrial laboratories to do everything in their power to encourage research work and to finance adequately the research programs outlined by the research staff, for they have opportunity to study problems that are seldom attacked by university workers because of lack of funds and physical facilities. They are doing a fine job in this regard, but I ask them to go still farther in the development of their reserach program. It will pay in satisfaction, as well as in dollars and cents.

Here is some doggerel which I have written to summarize the vitamin situation to date:

Vitamins Go to College

to question.

Vitamin D research has shown no pronounced trends in past months other than the tendency to show increased interest in activated 7-dehydrocholesterol as a commercial source of vitamin D for food fortification. With the exception of poultry, activated ergosterol and natural vitamin D seem to compare quite faorably for all practical purposes and can be used more or less interchangeably.

The chemistry of vitamin K, the antihemorrhagic vitamin, has been worked out and a number of related compounds have been found to promote prothrombin formation and hasten blood clotting. Clinical applications continue to appear in the medical literature, and a water soluble form of vitamin K has been described which can now be injected into the blood stream. Apparently, alfalfa leaf meal and putrefying fish continue to be the principal sources of the K vitamins, of which there are two, K_1 and K_2.

Discussion of grass juice factors, gizzard erosion factors, factors U, W and H, lactation factors, etc., will be omitted here because chemists know very little about them.

MISCELLANEOUS PROBLEMS

Since I have devoted considerable time in past years to dairy and livestock problems, I have attempted to follow certain types of researches that are related to these fields.

Dr. Kon and co-workers in England at the National Dairy Research Institute have probably done the most comprehensive work on factors affecting the vitamin content of milk. They have recently restudied the effect of heating and processing of milk. They have described experiments in which milk was flash-heated at 160° F., homogenized, autoclaved at 212° F., the bottles closed and the heating continued to 230° F., and cooled, giving the milk plenty of punishment. They concluded that such treatment did not reduce digestibility, but they felt that the biological value of the proteins had been reduced about 6%. Carotene was not affected (this agrees with our own work) and storage seemed to have no deleterious effect on the A potency. B_1, or thiamin, however, was partly destroyed. Pasteurization destroyed but 10% of the thiamin, while sterilization accounted for about 50%. Riboflavin was found to be quite stable and was not affected appreciably. Vitamin C losses, on the other hand, amounted to as much as 40%, and the losses seemed to continue during storage.

Drying of milk was less destructive of thiamin than the evaporation, while vitamin A potency of milks stood up well under both methods of processing. Spray drying was less destructive of vitamin C than roller drying, the percentages of loss being 20% and 30%, respectively. Sterilized evaporated milks contained less vitamin C than dried milks. This is due, in all probability, to the fact that copper pans were used. It has been my experience that heat, properly applied, in the absence of oxygen and copper, leads to satisfactory preservation of vitamin C in milk. Light is also harmful in the presence of certain catalysts, one of which may be riboflavin.

Kon and his co-workers have also restudied the old question of the nutritive

casionally experience. In other words, the Jersey and the Guernsey are less efficient as "carotene converters." The poorer pigmenters, like the Holsteins and Ayrshires, may and often do eat more carotene per day, yet they invariably store and excrete less carotene. Our work shows that the Holstein is a better converter of carotene. As a result, the Holstein stores and excretes a greater amount of the more or less colorless sterol, vitamin A, and less of the orange yellow hydrocarbon pigment, carotene. Gram for gram and pound for pound, butterfats from all these breeds display about the same total biological vitamin A potency when fed to animals. Therefore, butterfats from pigmented breeds are richer in untransformed carotene and poorer in the transformed vitamin A. Conversely, the less pigmented breeds yield butterfats with less carotene but more vitamin A. This means that the unstandardized milks rich in butterfat will contain more vitamin A units or potency per quart than low-fat milks, providing the cows are fed the same ration.

Of all the carotenoid pigments in feeds, the prevailing pigment which passes into the butterfat is beta carotene. If carrots are fed to cows in quantity, alpha carotene is also found in the fat. It is interesting to note that the red pigment, lycopene, found in tomatoes, cannot pass into the butterfat. This red pigment is an isomeric hydrocarbon very similar in structure to carotene. Apparently, the cow has no mechanism whereby she can convey this lycopene to milk. Where the barrier is and how lycopene is selectively excluded, are not known.

Hauge and his co-workers at Purdue state that the cow must receive more than 500,000 units of vitamin A (as carotene) per day in order to produce a butterfat of maximum A potency. These workers have also called attention to the existence of a substance in soybeans which prevents transference of vitamin A from the feed to the milk. They believe the substance is thermostable and that it can be removed by adsorption on Nuchar. It is said to be present in both the bean and the oil. This work will, of course, have to be confirmed before it is definitely accepted.

The question of iodized milks appears in scientific literature from time to time. A recent paper from Ohio by Mathews, Curtis and Meyers states that the feeding of potassium iodide increased the iodine content of milk from 7 to 26 times that of ordinary milk.

The riboflavin content of milk is a live question at present. Sharp and co-workers at Cornell feel that riboflavin can be increased to 3.5 milligrams per liter by feeding. Others doubt whether this can be done. Whitnah and co-workers at Kansas believe that breed differences exist, ranging from 1.17 milligrams per liter in Ayrshire milk to 1.73 milligrams per liter in Jersey milk. Sharp reports an average of 2 milligrams per liter in the Cornell dairy herd. So far as our own dairy herd is concerned, we find about 1.5 milligrams of riboflavin per quart.

Dr. Hunt and his group at Ohio, and others, have confirmed our early work, with Dr. Bechdel, of several years ago, relative to the synthesis of the vitamin B

venjem and others have advanced evidence to show that thiamin, riboflavin, nicotinic acid, pyridoxine (B_6), pantothenic acid, biotin and vitamin K are all synthesized in the rumen.

Kon of England maintains that sprouted corn caused increased excretion of ascorbic acid (vitamin C) in cows' milk. As early as 1919, our laboratory, as well as those of Hart at Wisconsin and Hess and Supplee in New York, drew similar conclusions regarding the effect of the vitamin C content of the ration on the vitamin C content of the milk. In the intervening years, many laboratories, started by the work of Hughes at Kansas, have questioned the ability of the ration to change the vitamin C content of milk appreciably. Holmes, in a recent paper, finds seasonal fluctuations in the ascorbic acid content of milk, but doubts whether diet or period of lactation have much effect.

We at Pennsylvania have restudied the question recently, using chemical estimations of ascorbic acid, and the old work of 20 years ago is, of course, open to question, since guinea pigs were evidently responding to milk vitamins which at that time had not been discovered. Recently, we restudied the whole situation.

Our first problem, of course, was the collection of milk, blood and urine in order to find out how to estimate the ascorbic acid accurately. We finally satisfied ourselves on the ascorbic acid determination in blood, milk and urine, using the dye titration method, and the Roe furfuraldehyde method.

We used only Holstein cows, to eliminate any variations due to breed, and we fed the largest doses of ascorbic acid or vitamin C that have ever been recorded. We have fed as high as 4,000,000 international units per day, or nearly ½ lb of ascorbic acid in pure crystalline form. Of course, we couldn't inject that much, but we have injected as much as 1,000,000 units per day.

We administered the ascorbic acid to cows in five different ways: (1) by feeding with small amounts of corn silage, (2) by drenching from a bottle, (3) by intravenous injection, (4) by subcutaneous injection, and (5) with gelatin capsules.

We were unable to find an increase in the ascorbic acid content of the blood, the milk or the urine by feeding methods. When we injected ascorbic acid subcutaneously and intravenously, we got a slight increase of ascorbic acid in the blood and we observed an increased output in urine and milk. We could account, in some of the experiments, for as high as 90% of the total ascorbic acid injected. However, by the gelatin capsule method, we got the same results as we did by the other feeding methods. The question arises: Where is it going and where was it destroyed?

We produced a rumen fistula and studied the question with the opportunity of taking samples from the rumen. We found that vitamin C is very rapidly destroyed in the rumen. We then removed the rumen contents and put them in beakers and studied the speed of ascorbic acid destruction in vivo as well as in vitro. We added known quantities of ascorbic acid to the beakers, and got practically the same results as were ob-

ascorbic acid.

We have been interested in the last three or four years in the effect of exercise on vitamin requirement. We have divided our rats into three groups: one group gets no exercise, another group is permitted to exercise voluntarily, and yet another group is forced to exercise by having the rotor run by a motor at the rate of about 1,000 revolutions per day.

Briefly, we found that the fat, lazy rats that got no exercise came down with vitamin A deficiency faster than those that were forced to exercise or which exercised voluntarily. We also found that the exercised rats had better intestinal motility as measured by number and size of fecal pellets.

The work done by Dr. Hunter and co-workers on the vitamin requirements of poultry, as well as by Bechdel and co-workers on the vitamin D requirements of dairy cattle and that of Guilbert and Hart on the vitamin requirements of horses and other forms of livestock, is very important and should be continued. Further data must also be obtained relative to the vitamin requirements of human beings in health and disease.

Twenty years ago, one of my students decided to go into another field of work because he thought that all vitamin problems had been solved. Since that time the whole vitamin field has undergone unbelievable transformations. With all the knowledge we have to date, the field is hardly scratched. Each new discovery opens up hundreds of new problems.

I appeal to the executive officers of the various industrial laboratories to do everything in their power to encourage research work and to finance adequately the research programs outlined by the research staff, for they have opportunity to study problems that are seldom attacked by university workers because of lack of funds and physical facilities. They are doing a fine job in this regard, but I ask them to go still farther in the development of their reserach program. It will pay in satisfaction, as well as in dollars and cents.

Here is some doggerel which I have written to summarize the vitamin situation to date:

Vitamins Go to College

Vitamin A is a sterol, they say.
  If it's absent? The thought makes me shiver!
But now it would seem, if I eat carotene,
  I can make the darn thing in my liver.
B_1, thiamin, and B_2, a flavin,
  Play a chemical role, enzymatic;
B's 3, 4 and 5 have gone back to the hive,
  Of which I approve most emphatic.
B_6 (pyridoxine) acts like antitoxine,
  Curing rats with sore paws and red skins;
Nicotinic, the acid, now makes people placid,
  When once they have been pellagrins.
Some folks react splenic, without pantothenic,
  Because their dark hair starts to gray;
Without Cevitamic, they lie in a hammock
  And take pills and pellets all day.
And now, if you please, they say we've two D's,
  That we need to cure rickets and jitters;
And E, a sterol (alphatocopherol),
  Causes rats to produce many litters.
Vitamin F is ignored by the chef,
  When you're fat and your tissues are flaccid;
But rats become thin with a rough, inflamed skin,
  Through the lack of linoleic acid.
You may be a good feeder and still be a bleeder,
  If you lack blood prothrombin, they say,
But it makes you feel swell to know you'll get well
  If the doctor has Vitamin K.

l'envoi

I don't mind addressing the drug store clerk
  In vitamin terms alphabetical,
But it sure is terrific to be scientific,
  Using three-dollar terms, hypothetical.

REDUCTION
CHANGE
10 X TO 14 X

power to a pure chemical compound, para amino benzoic acid, but his results as yet have not been confirmed by others.

Vitamin B_6, pyridoxine, is now obtainable in crystalline form, and its chemical formula can be written. While it is known as the anti-acrodynia factor, it is not of sufficient practical importance at present to devote much time to it.

OTHER FACTORS

Alpha tocopherol, an alcohol, is also known as vitamin E. It can be manufactured in quantity by microdistillation and seems to function other than as an "anti-sterility" vitamin. Apparently, it plays an important role in preventing and curing certain types of muscular dystrophy and has been described as preventing encephalomalacia in "crazy chicks." Its clinical value is still open to question.

Vitamin D research has shown no pronounced trends in past months other than the tendency to show increased interest in activated 7-dehydrocholesterol as a commercial source of vitamin D for food fortification. With the exception of poultry, activated ergosterol and natural vitamin D seem to compare quite faorably for all practical purposes and can be used more or less interchangeably.

The chemistry of vitamin K, the anti-hemorrhagic vitamin, has been worked out and a number of related compounds have been found to promote prothrombin formation and hasten blood clotting. Clinical applications continue to appear in the medical literature, and a water soluble form of vitamin K has been described which can now be injected into the blood stream. Apparently, alfalfa leaf meal and putrefying fish continue to be the principal sources of the K vitamins, of which there are two, K_1 and K_2.

Discussion of grass juice factors, gizzard erosion factors, factors U, W and H, lactation factors, etc., will be omitted here because chemists know very little about them.

MISCELLANEOUS PROBLEMS

Since I have devoted considerable time in past years to dairy and livestock problems, I have attempted to follow certain types of researches that are related to these fields.

Dr. Kon and co-workers in England at the National Dairy Research Institute have probably done the most comprehensive work on factors affecting the vitamin content of milk. They have recently restudied the effect of heating and processing of milk. They have described experiments in which milk was flash-heated at 160° F., homogenized, autoclaved at 212° F., the bottles closed and the heating continued to 230° F., and cooled, giving the milk plenty of punishment. They concluded that such treatment did not reduce digestibility, but they felt that the biological value of the proteins had been reduced about 6%. Carotene was not affected (this agrees with our own work) and storage seemed to have no deleterious effect on the A potency. B_1, or thiamin, however, was partly destroyed. Pasteurization destroyed but 10% of the thiamin, while sterilization accounted for about 50%. Riboflavin was found to be quite stable and was not affected appreciably. Vitamin C losses, on the other hand, amounted to as much as 40%, and the losses seemed to continue during storage.

Drying of milk was less destructive of thiamin than the evaporation, while vitamin A potency of milks stood up well under both methods of processing. Spray drying was less destructive of vitamin C than roller drying, the percentages of loss being 20% and 30%, respectively. Sterilized evaporated milks contained less vitamin C than dried milks. This is due, in all probability, to the fact that copper pans were used. It has been my experience that heat, properly applied, in the absence of oxygen and copper, leads to satisfactory preservation of vitamin C in milk. Light is also harmful in the presence of certain catalysts, one of which may be riboflavin.

Kon and his co-workers have also restudied the old question of the nutritive value of raw versus pasteurized milk. Their work with calves and human beings agrees with most other work of similar nature; namely, that no outstanding differences can be noted from a practical standpoint.

The effect of diet on the nutritive value of milk continues to hold the attention of many workers, including ourselves, at Pennsylvania State College. It is now well established that the vitamin A potency of milk is related directly to the carotene content of the ration. Various laboratories over the country have shown that the "pigmented breeds," or Jerseys and Guernseys, can be made to produce practically colorless butterfat if fed for a sufficient length of time on a carotene-free diet. It was concluded many years ago that the so-called pigmented breeds are, in a sense, pathological, going through life with incipient carotinemia, such as human beings occasionally experience. In other words, the Jersey and the Guernsey are less efficient as "carotene converters." The poorer pigmenters, like the Holsteins and Ayrshires, may and often do eat more carotene per day, yet they invariably store and excrete less carotene. Our work shows that the Holstein is a better converter of carotene. As a result, the Holstein stores and excretes a greater amount of the more or less colorless sterol, vitamin A, and less of the orange yellow hydrocarbon pigment, carotene. Gram for gram and pound for pound, butterfats from all these breeds display about the same total biological vitamin A potency when fed to animals. Therefore, butterfats from pigmented breeds are richer in untransformed carotene and poorer in the transformed vitamin A. Conversely, the less pigmented breeds yield butterfats with less carotene but more vitamin A. This means that the unstandardized milks rich in butterfat will contain more vitamin A units or potency per quart than low-fat milks, providing the cows are fed the same ration.

Of all the carotenoid pigments in feeds, the prevailing pigment which passes into the butterfat is beta carotene. If carrots are fed to cows in quantity, alpha carotene is also found in the fat. It is interesting to note that the red pigment, lycopene, found in tomatoes, cannot pass into the butterfat. This red pigment is an isomeric hydrocarbon very similar in structure to carotene. Apparently, the cow has no mechanism whereby she can convey this lycopene to milk. Where the barrier is and how lycopene is selectively excluded, are not known.

Hauge and his co-workers at Purdue state that the cow must receive more than 500,000 units of vitamin A (as carotene) per day in order to produce a butterfat of maximum A potency. These workers have also called attention to the existence of a substance in soybeans which prevents transference of vitamin A from the feed to the milk. They believe the substance is thermostable and that it can be removed by adsorption on Nuchar. It is said to be present in both the bean and the oil. This work will, of course, have to be confirmed before it is definitely accepted.

The question of iodized milks appears in scientific literature from time to time. A recent paper from Ohio by Mathews, Curtis and Meyers states that the feeding of potassium iodide increased the iodine content of milk from 7 to 26 times that of ordinary milk.

The riboflavin content of milk is a live question at present. Sharp and co-workers at Cornell feel that riboflavin can be increased to 3.5 milligrams per liter by feeding. Others doubt whether this can be done. Whitnah and co-workers at Kansas believe that breed differences exist, ranging from 1.17 milligrams per liter in Ayrshire milk to 1.73 milligrams per liter in Jersey milk. Sharp reports an average of 2 milligrams per liter in the Cornell dairy herd. So far as our own dairy herd is concerned, we find about 1.5 milligrams of riboflavin per quart.

Dr. Hunt and his group at Ohio, and others, have confirmed our early work, with Dr. Bechdel, of several years ago, relative to the synthesis of the vitamin B complex in the rumen of the dairy cow by micro-organisms. Hunt, with more recent and refined techniques, has found that riboflavin is synthesized in the rumen of the cow in appreciable quantities. This has been our feeling ever since riboflavin was recognized, because our synthetic organism was a yellow pigmenter, which Professor Knutsen named "flavo-bacterium vitarumen."

Hunt doubts whether vitamin B_1 is synthesized in the cow's rumen, while McElroy and Goss of California believe they have proved it to be synthesized in the rumen of sheep. These workers could not prove that it is synthesized in the cow's rumen, although the milk contained normal quantities. A non-fistulated cow did have thiamin in the rumen and they conclude, as we at Pennsylvania did, that B_1 is probably not a dietary essential for ruminants, so far as the feed is concerned. Recently, Hart, Elvehjem and others have advanced evidence to show that thiamin, riboflavin, nicotinic acid, pyridoxine (B_6), pantothenic acid, biotin and vitamin K are all synthesized in the rumen.

Kon of England maintains that sprouted corn caused increased excretion of ascorbic acid (vitamin C) in cows' milk. As early as 1919, our laboratory, as well as those of Hart at Wisconsin and Hess and Supplee in New York, drew similar conclusions regarding the effect of the vitamin C content of the ration on the vitamin C content of the milk. In the intervening years, many laboratories, started by the work of Hughes at Kansas, have questioned the ability of the ration to change the vitamin C content of milk appreciably. Holmes, in a recent paper, finds seasonal fluctuations in the ascorbic acid content of milk, but doubts whether diet or period of lactation have much effect.

We at Pennsylvania have restudied the question recently, using chemical estimations of ascorbic acid, and the old work of 20 years ago is, of course, open to question, since guinea pigs were evidently responding to milk vitamins which at that time had not been discovered. Recently, we restudied the whole situation.

Our first problem, of course, was the collection of milk, blood and urine in order to find out how to estimate the ascorbic acid accurately. We finally satisfied ourselves on the ascorbic acid determination in blood, milk and urine, using the dye titration method, and the Roe furfuraldehyde method.

We used only Holstein cows, to eliminate any variations due to breed, and we fed the largest doses of ascorbic acid or vitamin C that have ever been recorded. We have fed as high as 4,000,000 international units per day, or nearly ½ lb of ascorbic acid in pure crystalline form. Of course, we couldn't inject that much, but we have injected as much as 1,000,000 units per day.

We administered the ascorbic acid to cows in five different ways: (1) by feeding with small amounts of corn silage, (2) by drenching from a bottle, (3) by intravenous injection, (4) by subcutaneous injection, and (5) with gelatin capsules.

We were unable to find an increase in the ascorbic acid content of the blood, the milk or the urine by feeding methods. When we injected ascorbic acid subcutaneously and intravenously, we got a slight increase of ascorbic acid in the blood and we observed an increased output in urine and milk. We could account, in some of the experiments, for as high as 90% of the total ascorbic acid injected. However, by the gelatin capsule method, we got the same results as we did by the other feeding methods. The question arises: Where is it going and where was it destroyed?

We produced a rumen fistula and studied the question with the opportunity of taking samples from the rumen. We found that vitamin C is very rapidly destroyed in the rumen. We then removed the rumen contents and put them in beakers and studied the speed of ascorbic acid destruction in vivo as well as in vitro. We added known quantities of ascorbic acid to the beakers, and got practically the same results as were obtained in the rumen, except that the destruction time was a little longer. Body temperature and aeration through the churning of the rumen contents had much to do with the destruction.

We are now studying the mechanism of this destruction in the rumen. Also, we have evidence that the cow is capable of synthesizing vitamin C.

We are also planning some long-time experiments on vitamin C-free diets, to see just how far we can go in reducing the ascorbic acid content of milk by feeding. We have evidence that we can go as low as 15 milligrams per quart and as high as 30 milligrams per quart by feeding, but we do not yet know if these are the absolute limits.

Whitnah and Ridell at Kansas state that they are able to obtain increases in blood ascorbic acid by feeding fresh rye. We want to repeat their work and compare it with the various sources of ascorbic acid.

We have been interested in the last three or four years in the effect of exercise on vitamin requirement. We have divided our rats into three groups: one group gets no exercise, another group is permitted to exercise voluntarily, and yet another group is forced to exercise by having the rotor run by a motor at the rate of about 1,000 revolutions per day.

Briefly, we found that the fat, lazy rats that got no exercise came down with vitamin A deficiency faster than those that were forced to exercise or which exercised voluntarily. We also found that the exercised rats had better intestinal motility as measured by number and size of fecal pellets.

The work done by Dr. Hunter and co-workers on the vitamin requirements of poultry, as well as by Bechdel and co-workers on the vitamin D requirements of dairy cattle and that of Guilbert and Hart on the vitamin requirements of horses and other forms of livestock, is very important and should be continued. Further data must also be obtained relative to the vitamin requirements of human beings in health and disease.

Twenty years ago, one of my students decided to go into another field of work because he thought that all vitamin problems had been solved. Since that time the whole vitamin field has undergone unbelievable transformations. With all the knowledge we have to date, the field is hardly scratched. Each new discovery opens up hundreds of new problems.

I appeal to the executive officers of the various industrial laboratories to do everything in their power to encourage research work and to finance adequately the research programs outlined by the research staff, for they have opportunity to study problems that are seldom attacked by university workers because of lack of funds and physical facilities. They are doing a fine job in this regard, but I ask them to go still farther in the development of their reserach program. It will pay in satisfaction, as well as in dollars and cents.

Here is some doggerel which I have written to summarize the vitamin situation to date:

Vitamins Go to College

Vitamin A is a sterol, they say.
  If it's absent? The thought makes me shiver!
But now it would seem, if I eat carotene,
  I can make the darn thing in my liver.
B_1, thiamin, and B_2, a flavin,
  Play a chemical role, enzymatic;
B's 3, 4 and 5 have gone back to the hive,
  Of which I approve most emphatic.
B_6 (pyridoxine) acts like antitoxine,
  Curing rats with sore paws and red skins;
Nicotinic, the acid, now makes people placid,
  When once they have been pellagrins.
Some folks react splenic, without pantothenic,
  Because their dark hair starts to gray;
Without Cevitamic, they lie in a hammock
  And take pills and pellets all day.
And now, if you please, they say we've two D's,
  That we need to cure rickets and jitters;
And E, a sterol (alphatocopherol),
  Causes rats to produce many litters.
Vitamin F is ignored by the chef,
  When you're fat and your tissues are flaccid;
But rats become thin with a rough, inflamed skin,
  Through the lack of linoleic acid.
You may be a good feeder and still be a bleeder,
  If you lack blood prothrombin, they say,
But it makes you feel swell to know you'll get well
  If the doctor has Vitamin K.

l'envoi

I don't mind addressing the drug store clerk
  In vitamin terms alphabetical,
But it sure is terrific to be scientific,
  Using three-dollar terms, hypothetical.

pasteurized milk.
s and human beings
er work of similar
no outstanding dif-
d from a practical

on the nutritive
ies to hold the at-
ers, including our-
a State College. It
d that the vitamin
related directly to
f the ration. Vari-
the country have
nented breeds," or
s, can be made to
lorless butterfat if
ngth of time on a
It was concluded
the so-called pig-
in a sense, patho-
life with incipient
human beings oc-
In other words,
Guernsey are less
converters." The
ike the Holsteins
and often do eat
y, yet they invari-
less carotene. Our
Iolstein is a better
. As a result, the
excretes a greater
or less colorless
less of the orange
pigment, carotene.
pound for pound,
these breeds dis-
e total biological
en fed to animals.
from pigmented
transformed caro-
transformed vita-
the less pigmented
with less carotene
This means that
lks rich in butter-
vitamin A units
than low-fat milks,
are fed the same

pigments in feeds,
which passes into
carotene. If car-
in quantity, alpha
in the fat. It is
t the red pigment,
atoes, cannot pass
his red pigment is
on very similar in

complex in the rumen of the dairy cow by micro-organisms. Hunt, with more recent and refined techniques, has found that riboflavin is synthesized in the rumen of the cow in appreciable quantities. This has been our feeling ever since riboflavin was recognized, because our synthetic organism was a yellow pigmenter, which Professor Knutsen named "flavo-bacterium vitarumen."

Hunt doubts whether vitamin B_1 is synthesized in the cow's rumen, while McElroy and Goss of California believe they have proved it to be synthesized in the rumen of sheep. These workers could not prove that it is synthesized in the cow's rumen, although the milk contained normal quantities. A non-fistulated cow did have thiamin in the rumen and they conclude, as we at Pennsylvania did, that B_1 is probably not a dietary essential for ruminants, so far as the feed is concerned. Recently, Hart, Elvehjem and others have advanced evidence to show that thiamin, riboflavin, nicotinic acid, pyridoxine (B_6), pantothenic acid, biotin and vitamin K are all synthesized in the rumen.

Kon of England maintains that sprouted corn caused increased excretion of ascorbic acid (vitamin C) in cows' milk. As early as 1919, our laboratory, as well as those of Hart at Wisconsin and Hess and Supplee in New York, drew similar conclusions regarding the effect of the vitamin C content of the ration on the vitamin C content of the milk. In the intervening years, many laboratories, started by the work of Hughes at Kansas, have questioned the ability of the ration to change the vitamin C content of milk appreciably. Holmes, in a recent paper, finds seasonal fluctuations in the ascorbic acid content of milk, but doubts whether diet or period of lactation have much effect.

We at Pennsylvania have restudied the question recently, using chemical estimations of ascorbic acid, and the old work of 20 years ago is, of course, open to question, since guinea pigs were evidently responding to milk vitamins which at that time had not been discovered. Recently, we restudied the whole situation.

Our first problem, of course, was the collection of milk, blood and urine in order to find out how to estimate the ascorbic acid accurately. We finally satisfied ourselves on the ascorbic acid

tained in the rumen, except that the destruction time was a little longer. Body temperature and aeration through the churning of the rumen contents had much to do with the destruction.

We are now studying the mechanism of this destruction in the rumen. Also, we have evidence that the cow is capable of synthesizing vitamin C.

We are also planning some long-time experiments on vitamin C-free diets, to see just how far we can go in reducing the ascorbic acid content of milk by feeding. We have evidence that we can go as low as 15 milligrams per quart and as high as 30 milligrams per quart by feeding, but we do not yet know if these are the absolute limits.

Whitnah and Ridell at Kansas state that they are able to obtain increases in blood ascorbic acid by feeding fresh rye. We want to repeat their work and compare it with the various sources of ascorbic acid.

We have been interested in the last three or four years in the effect of exercise on vitamin requirement. We have divided our rats into three groups: one group gets no exercise, another group is permitted to exercise voluntarily, and yet another group is forced to exercise by having the rotor run by a motor at the rate of about 1,000 revolutions per day.

Briefly, we found that the fat, lazy rats that got no exercise came down with vitamin A deficiency faster than those that were forced to exercise or which exercised voluntarily. We also found that the exercised rats had better intestinal motility as measured by number and size of fecal pellets.

The work done by Dr. Hunter and co-workers on the vitamin requirements of poultry, as well as by Bechdel and co-workers on the vitamin D requirements of dairy cattle and that of Guilbert and Hart on the vitamin requirements of horses and other forms of livestock, is very important and should be continued. Further data must also be obtained relative to the vitamin requirements of human beings in health and disease.

Twenty years ago, one of my students decided to go into another field of work because he thought that all vitamin problems had been solved. Since that time the whole vitamin field has undergone unbelievable transformations. With

---

Pa.-68

THE DISTRIBUTION OF POTASSIUM IN BRIGHT LEAF CIGARETTE TOBACCO AND ITS INFLUENCE ON THE QUALITY OF THE LEAF

BY

M. F. GRIBBINS, J. J. REID, AND D. E. HALEY
(Contribution from Pennsylvania Agricultural Experiment Station)

Reprinted from **JOURNAL OF AGRICULTURAL RESEARCH**
Vol. 63, No. 1 : : : : : Washington, D. C., July 1, 1941
(Pages 31–39)

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

U. S. GOVERNMENT PRINTING OFFICE : 1941

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, Chairman
Chief, Bureau of Agricultural Chemistry and Engineering

JOHN W. ROBERTS
Principal Pathologist, Bureau of Plant Industry

BENJAMIN SCHWARTZ
Principal Zoologist, Chief, Zoological Division, Bureau of Animal Industry

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
Director of Research, Pennsylvania Agricultural Experiment Station

V. R. GARDNER
Director, Michigan Agricultural Experiment Station

C. B. HUTCHISON
Director, California Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:
Entire Journal: Domestic, $3.25 a year (2 volumes)
Foreign, $4.75 a year (2 volumes)
Single numbers: Domestic, 15 cents
Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. When purchased in quantity of 100 or more a reduction of 25 percent in the cost will be made. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

THE DISTRIBUTION OF POTASSIUM IN BRIGHT LEAF CIGARETTE TOBACCO AND ITS INFLUENCE ON THE QUALITY OF THE LEAF [1]

By M. F. Gribbins, *assistant in agricultural and biological chemistry*, J. J. Reid *assistant professor of bacteriology*, and D. E. Haley, *professor of soils and phytochemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Numerous workers have reported that potash fertilization of leaf tobacco is favorable both to the yield and quality of the crop. Many fundamental studies have been conducted on the absorption and translocation of potassium by growing plants and its accumulation within the various cells and tissues. The relation of this element to various biochemical and physiological phenomena has received due consideration.

The present investigations involve a study of the influence of various quantities of potash fertilizers applied to growing plants in the field on the absorption and localization of potassium within the plants and the influence of this element upon their quality. For these studies, samples of tobacco were obtained from certain of the experimental plots at Chatham, Va., where the Virginia Agricultural Experiment Station is conducting field tests on this crop. Samples of both the 1938 and 1939 crops were taken in December from a barn where the mature leaves had been stored in the usual manner after harvesting and curing. The results of studies conducted on similar samples of the 1937 crop have been reported (*2*).[2]

REVIEW OF THE LITERATURE

Within the growing plant potassium may be found in the form of compounds which are quite soluble in water. It is readily transported throughout the plant and is usually found to accumulate in regions of active growth. Nightingale, Schermerhorn, and Robbins (*6*) found that the greater part of the potassium in tomato plants was soluble in water and, when present in limited quantities, was translocated from regions of less intensive to regions of more intensive metabolism. If a potassium-deficient plant was allowed to fruit, the potassium was translocated to the fruit at the expense of all other portions of the plant, which resulted in its death. Confirmatory results have been reported by James and Penston (*3*) and by Janssen and Bartholomew (*4*). The last-named workers found that leaf starvation in the tomato plant due to a lack of potassium was progressive, and extended from the lower to the upper leaves. Dead leaves taken from the plants showed a much lower content of potassium than actively growing leaves, indicating a reutilization of this element in metabolism. It is apparent, therefore, that a plant should contain ample quantities of

---

[1] Received for publication August 31, 1940. Paper No. 990 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 38.

Journal of Agricultural Research, Washington, D. C.
Vol. 63, No. 1
July 1, 1941
Key No. Pa.-68

potassium for its normal growth and metabolism, and that there should be a fair distribution of this element throughout the plant.

MATERIALS USED AND METHODS OF ANALYSIS

The samples were taken from plots each of which received 30 pounds of nitrogen, 80 pounds of phosphoric acid, and various quantities of potash per acre as shown in table 1. Supplementary treatments involved the application of magnesia and limestone to all plots at the rate of 200 pounds per acre. Two percent of the potash was applied as muriate, the remainder as the sulfate. Representative samples[3] of leaves were taken from the lower, the middle, and the upper third of the stalks. Each sample represented two grades, good quality and scrap quality. In 1938 only the good quality grades were analyzed.

Methods used for chemical analysis involved the removal of the midrib and grinding the remainder in a Wiley mill. Total nitrogen was determined by the Kjeldahl method modified to include the nitrogen of nitrates, potassium by the platinic-chloride method according to Thomas et al. (*10*), and moisture by drying at 97.5° C. for 2 hours in a drying oven. Reducing materials were determined on an aqueous extract of the sample by the Quisumbing and Thomas method (*7*) and calculated as glucose. Samples were extracted with water at 50° C. for 2 hours and then overnight at room temperature. The samples were clarified, deleaded, made up to volume, filtered, and the reducing power determined on aliquots.

TABLE 1.—*Fertilizer treatment of the experimental tobacco plots studied*[1]

Plot No.	Row treatment, 1,000 pounds per acre of indicated formula	Side dressing of K_2O per acre[2]	Total K_2O in treatment, per acre
		Pounds	*Pounds*
1	3-8-0	0	0
2	3-8-3	0	30
3	3-8-6	0	60
4	3-8-6	30	90
5	3-8-6	60	120
6	3-8-6	100	160
7	3-8-6	140	200
8	3-8-6	240	300

[1] Data presented in this table were obtained from E. M. Matthews, superintendent of the Chatham substation of the Virginia Agricultural Experiment Station.

[2] Side dressings were applied on either side of the row about 8 inches from the plants 3 weeks after transplanting.

PRESENTATION OF DATA

Samples of both the 1938 and 1939 crops were analyzed for potassium, nitrogen, and reducing materials. The reducing materials, to a large extent, are made up of fermentable carbohydrates. A large portion, but by no means all, of these materials is made up of glucose and related sugars.

The 1938 crop produced good yields and commanded a fair price on the market (table 2), notwithstanding the fact that it was grown under conditions of excessive rainfall and was somewhat damaged by hail. The analyses made on the best quality samples are presented in table 3.

---

[3] These samples were submitted through the courtesy of E. M. Matthews, superintendent of the Chatham substation.

TABLE 2.—*Effect of various fertilizer treatments on the yield and value of the 1938 and 1939 crops of bright leaf tobacco produced on the experimental plots studied*

1938 CROP

Plot No.[1]	Yield per acre	Value per acre	Price per pound
	Pounds	*Dollars*	*Cents*
1	758	140.20	18.5
2	1,096	218.08	19.9
3	1,168	239.70	20.5
4	1,192	254.35	21.3
5	1,188	233.63	19.7
6	1,222	241.09	19.7
7	1,198	222.74	18.6
8	1,228	237.53	19.3

1939 CROP

Plot No.[1]	Yield per acre	Value per acre	Price per pound
1	888	120.40	13.6
2	1,096	150.80	13.8
3	1,112	155.60	14.0
4	1,104	162.00	14.7
5	1,084	147.60	13.6
6	1,120	156.40	14.0
7	1,148	143.60	12.5
8	1,076	144.80	13.5

[1] See table 1 for formulas and quantities of fertilizers used on these plots.

The 1939 crop was grown under dry conditions early in the season but later the rainfall was satisfactory. On the whole, however, this crop was somewhat inferior to that of the 1938 crop (table 2).

Chemical analyses of the tobacco for both years are presented in table 3, all results being reported on a moisture-free basis. From the results as listed in table 3, the ratio of nitrogen to potassium was calculated. The results are presented in figures 1 and 2.

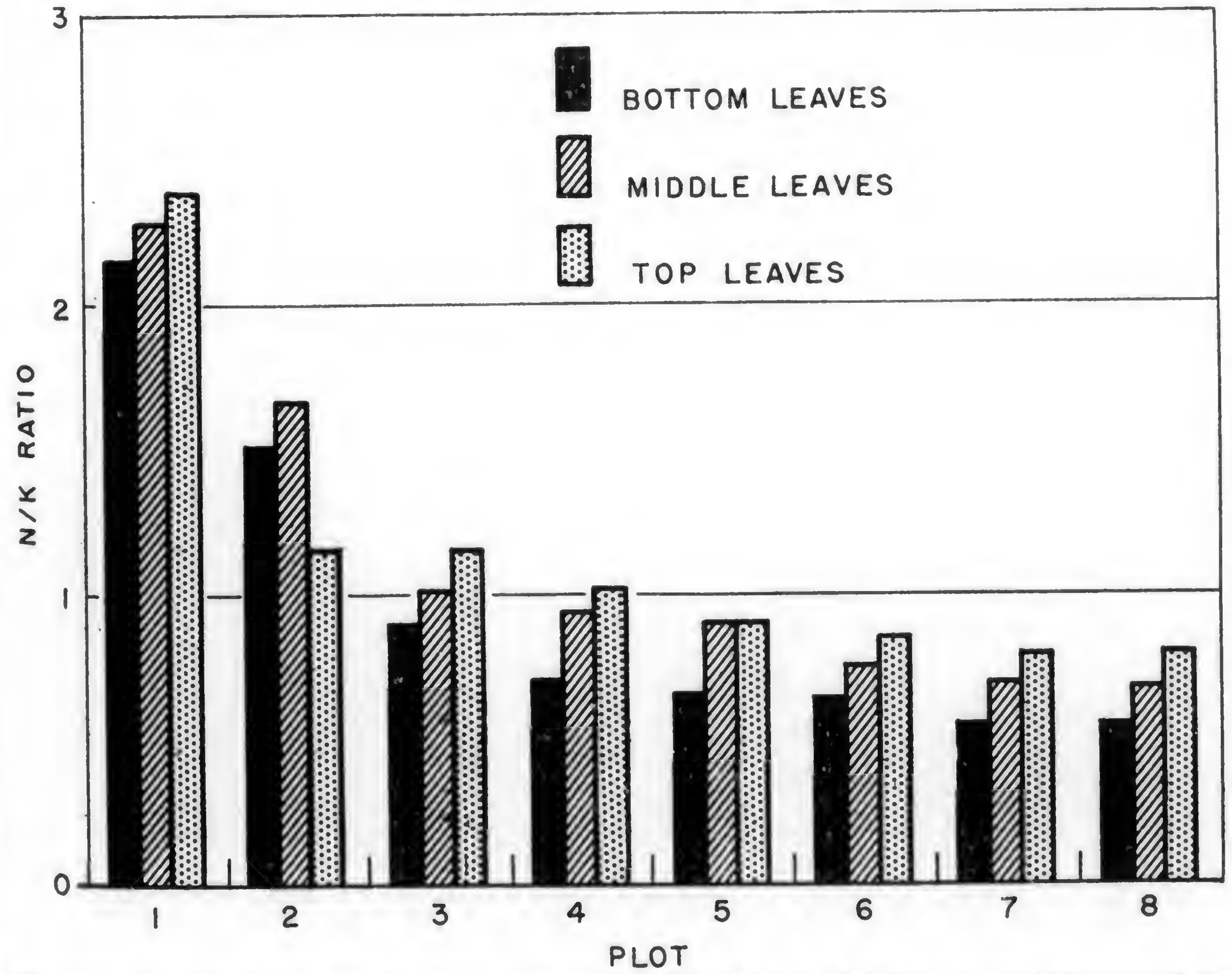


FIGURE 1.—The nitrogen/potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1938 crop, best quality.

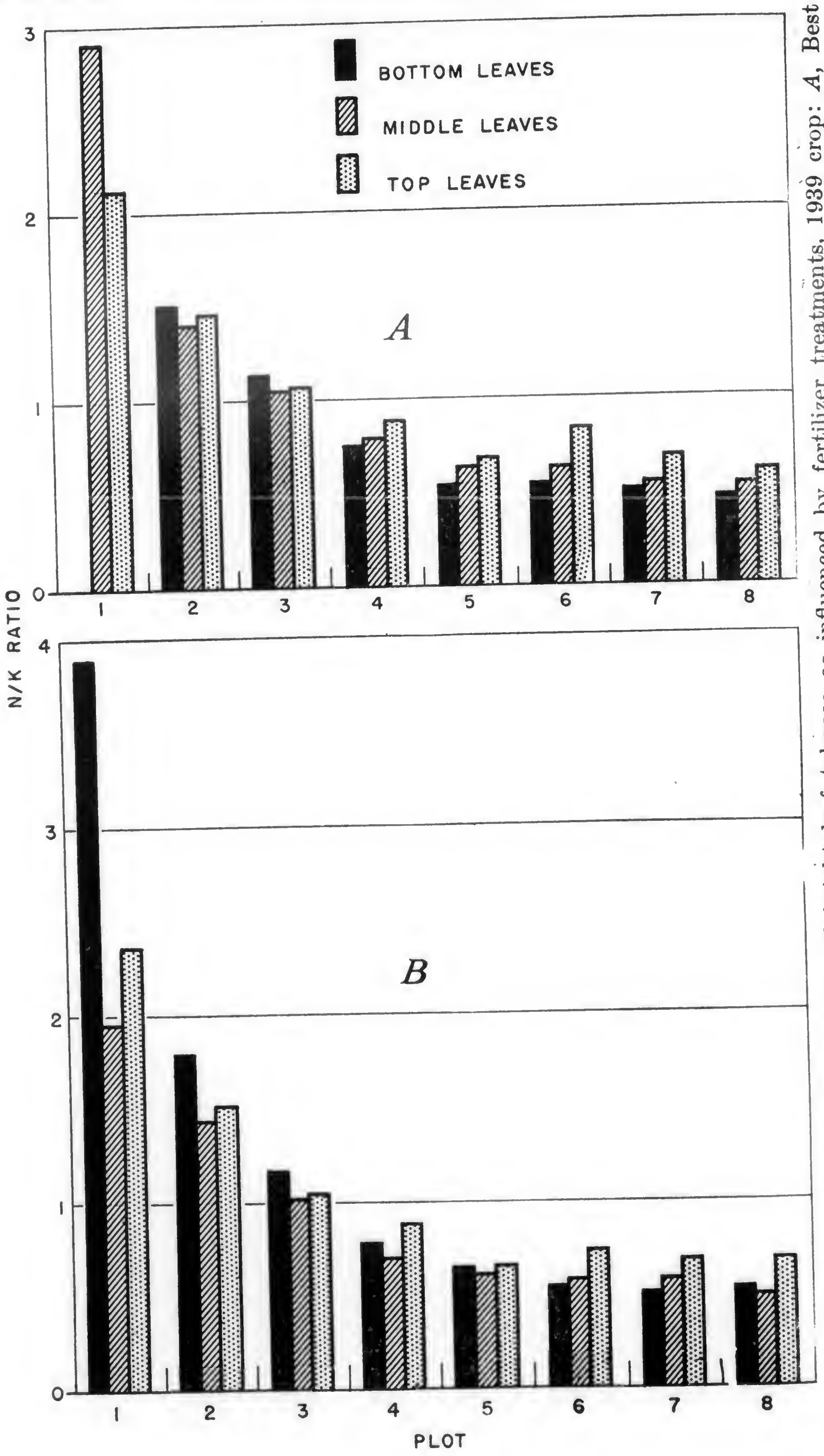


FIGURE 2.—The nitrogen/potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1939 crop: *A*, Best quality; *B*, scrap quality.

TABLE 3.—*Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*

1938, BEST QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	22.47	1.97	0.91	2.16	11.40
	Middle	13.48	2.60	1.14	2.28	5.18
	Top	5.87	3.22	1.35	2.38	1.83
2	Bottom	23.65	1.79	1.18	1.52	13.20
	Middle	20.06	2.11	1.26	1.67	9.50
	Top	24.76	2.20	1.92	1.15	6.70
3	Bottom	24.03	1.71	1.91	.90	14.05
	Middle	21.83	1.95	1.93	1.01	11.19
	Top	15.43	2.31	2.00	1.15	6.68
4	Bottom	27.94	1.59	2.20	.72	17.57
	Middle	22.18	2.07	2.17	.95	10.72
	Top	18.60	2.15	2.11	1.02	8.65
5	Bottom	27.69	1.63	2.46	.66	16.98
	Middle	24.45	2.09	2.29	.91	11.68
	Top	14.18	2.35	2.59	.91	6.03
6	Bottom	28.42	1.71	2.68	.64	16.62
	Middle	22.63	1.86	2.44	.76	12.17
	Top	13.35	2.32	2.71	.85	5.75
7	Bottom	24.93	1.77	3.18	.56	14.08
	Middle	20.89	1.98	2.81	.70	10.55
	Top	18.62	2.21	2.78	.79	8.44
8	Bottom	22.40	1.80	3.24	.56	12.44
	Middle	25.29	1.85	2.72	.68	13.68
	Top	18.19	2.20	2.74	.80	8.27

1939, BEST QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
1	Bottom	----	----	----	----	----
	Middle	14.00	2.12	0.73	2.91	6.61
	Top	6.54	2.74	1.29	2.12	2.39
2	Bottom	14.15	2.10	1.39	1.51	6.74
	Middle	17.21	2.05	1.46	1.40	8.40
	Top	12.46	2.20	1.51	1.46	5.66
3	Bottom	12.08	2.06	1.82	1.13	5.87
	Middle	17.75	1.84	1.75	1.05	9.65
	Top	9.69	2.31	2.15	1.07	4.19
4	Bottom	13.70	2.21	2.91	.76	6.20
	Middle	22.58	1.93	2.43	.79	11.71
	Top	11.02	2.27	2.57	.88	4.86
5	Bottom	12.24	2.05	3.70	.55	5.98
	Middle	20.09	1.91	3.05	.63	10.51
	Top	8.93	2.36	3.48	.68	3.78
6	Bottom	13.21	2.09	3.82	.55	6.33
	Middle	21.50	1.89	2.99	.63	11.37
	Top	13.60	2.21	2.65	.83	6.15
7	Bottom	14.70	1.95	3.80	.51	7.55
	Middle	20.62	1.92	3.50	.55	10.73
	Top	10.17	2.33	3.38	.69	4.36
8	Bottom	16.85	1.88	4.08	.46	8.96
	Middle	20.97	1.86	3.44	.54	11.30
	Top	9.59	2.21	3.54	.62	4.34

TABLE 3.—*Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*—Con.

1939, SCRAP QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	6.70	2.37	0.61	3.88	2.82
	Middle	12.48	2.13	1.09	1.96	5.86
	Top	3.70	3.12	1.32	2.36	1.19
2	Bottom	7.57	2.27	1.27	1.79	3.33
	Middle	17.22	1.93	1.35	1.43	8.93
	Top	9.12	2.44	1.61	1.52	3.74
3	Bottom	5.54	2.28	1.97	1.16	2.43
	Middle	14.39	1.92	1.89	1.02	7.50
	Top	7.49	2.54	2.44	1.04	2.95
4	Bottom	5.81	2.39	3.08	.78	2.43
	Middle	21.05	1.72	2.44	.71	12.25
	Top	9.86	2.23	2.55	.88	4.42
5	Bottom	7.83	2.16	3.33	.65	3.62
	Middle	16.31	1.98	3.17	.62	8.25
	Top	7.81	2.24	3.44	.65	3.49
6	Bottom	5.07	2.42	4.30	.56	2.09
	Middle	18.05	1.90	3.30	.58	9.51
	Top	9.46	2.41	3.22	.75	3.92
7	Bottom	7.72	2.34	4.53	.52	3.30
	Middle	16.74	1.96	3.34	.59	8.55
	Top	8.35	2.39	3.50	.68	3.49
8	Bottom	8.03	2.23	4.17	.54	3.60
	Middle	17.04	1.72	3.58	.48	9.92
	Top	7.08	2.36	3.48	.68	3.00

[1] See table 1 for formulas and quantities of fertilizers used on these plots.

DISCUSSION OF RESULTS

The results on the intake and accumulation of potassium as indicated in table 3 are somewhat in agreement with the observations of Anderson and coworkers (*1*), who stated that where the supply of potash in the soil was quite low, this element was found in higher concentrations in the upper rather than in the lower leaves. On the other hand, where the supply was ample, there was a higher accumulation in the lower leaves.

That the distribution of potassium in the plant is influenced in a measure by the quantities available is indicated by table 3. In plots 1 and 2 the low level of available potassium appeared insufficient to meet the demands of the growing plant. Under these conditions a larger accumulation of this element should be expected in the upper portions. However, where ample or rather excessive quantities of potassium were available to the plants, it appeared to be absorbed throughout the growth period, resulting in a marked accumulation of this element in the lower plant parts.

The incorporation of relatively large quantities of potassium in the fertilizer mixtures, improperly balanced with other necessary mineral nutrients, does not appear to be advisable, since the market value of the tobacco produced on plots so treated was found to be quite low. The conditions under which these plants were grown, however, indicate that quantities approximating 100 pounds per acre may be desirable.

While it is not advisable to produce bright leaf tobacco containing large quantities of nitrogen, this element must be given due consideration as supplying certain properties to the leaves which are extremely desirable from the standpoint of both yield and quality. From the results obtained, the writers are inclined to believe that the ratio of nitrogen to potassium should approximate 1:1 in the leaves. (See figs. 1 and 2.) It is apparent that the plots receiving minimum quantities of potassium had a wider ratio, while those receiving excessive quantities had a narrower ratio than this value. Leaves showing a ratio of approximately 1:1 were found on the middle leaves of the plants grown on plots 3, 4, and 5 during the 1938 season.

It would appear that there is some correlation between the nitrogen/potassium ratio of the leaves and the fertilizer treatment of the plants. While the 1938 and 1939 crops were produced under different weather conditions, the figures for the nitrogen/potassium ratio were of somewhat the same order.

Samples of the 1938 crop showed an increase in the concentration of nitrogen from the lower to the upper leaves. However, there appeared to be a slight variation where the potassium supply was more or less ample. On the other hand, the variation was quite pronounced for plot 1 where no potassium was applied. This was likewise noted for samples taken from this plot in 1939, while the remaining plots showed approximately the same concentration of nitrogen in the bottom and top leaves, being somewhat lower in the middle leaves.

It has been stated on numerous occasions that normal carbohydrate formation is closely associated with the presence of ample quantities of potassium (*9, 8, 5*). While the quantity of available soil potassium in plot 1 may have been sufficient to meet the needs of the plants during their early period of growth, it apparently was not sufficient to meet their demands during the latter stages of growth, as may be noted in the very low concentration of carbohydrates in the mature plants.

The results presented for the 1938 crop in table 3 are of particular interest. The samples from practically all plots showed a larger quantity of reducing materials in the lower than in the higher leaves. For those plots receiving sufficient potash this difference was less pronounced.

It is quite apparent that the reducing materials of the leaf are more or less associated with its quality. A comparison of the composition of the two grades of tobacco obtained from the 1939 crop, as given in table 3, is striking. In all cases, the reducing materials of the bottom and top leaves of the scrap quality was low, while that of the middle leaves was somewhat higher. It may be noted that while the bottom leaves of samples from plot 4 had approximately the same nitrogen/potassium ratio, the reducing materials of the scrap quality were less than half that of the best quality tobacco.

Harvesting of bright leaf tobacco is accomplished by the removal of two or three leaves at a time as they become "ripe," starting at the bottom and progressing upward. This procedure is called "priming" and in a normal season five or more primings are taken from each plant. It is obvious, therefore, that weather conditions prior to a priming may influence the composition of the leaves. The moisture content of the soil has a great deal to do with the absorption of mineral nutrients by plants and in this connection it should be remembered that above-normal rainfall was experienced in 1938 while rainfall was below normal in 1939.

SUMMARY AND CONCLUSIONS

Samples of bright leaf cigarette tobacco were obtained from each of eight experimental plots where potash was the only variable in the individual fertilizer treatments, ranging from none to 300 pounds per acre. Samples were taken from both the 1938 and 1939 crops and divided into three groups according to their position on the stalk, as bottom, middle, and top leaves. Only the best quality samples of the 1938 crop were analyzed, but in 1939 analyses were made of both scrap and best quality. From the results obtained it may be concluded:

(1) When the quantity of potassium available to the plants is low, a greater concentration of this element occurs in the upper leaves, and the content of reducing materials, including glucose, is quite low in all the leaves.

(2) When the quantity of potash at the disposal of the plants is satisfactory, there is a more or less uniform concentration of this element in the bottom, middle, and top leaves and the production of reducing materials attains a maximum. During the season of 1938 these conditions prevailed where potash was applied at the rate of 90 pounds per acre.

(3) Extremely large applications of potash give rise to an increased absorption of this element, with a larger accumulation in the bottom leaves. Under the conditions of these experiments, such applications of potash, improperly balanced in respect to other nutrient materials, failed to show any further favorable influence on the production of reducing materials than that observed in plants receiving adequate potash fertilization.

(4) Under the conditions of these experiments, a nitrogen/potassium ratio of from 0.8 to 1.1 appears to be highly desirable. Leaves of tobacco plants grown under these varied conditions showed a nitrogen/potassium ratio ranging from 0.5 to 3.9. Neither extreme was found to be associated with good quality.

Desirable nitrogen/potassium ratios were obtained in 1938 from applications of from 60 to 120 pounds of potash, while desirable nitrogen/potassium ratios were obtained in 1939 from 60 to 90 pounds of potash applied on the basis of 1 acre, which may be attributed in large measure to seasonal differences.

LITERATURE CITED

(1) Anderson, P. J., Swanback, T. R., and Street, O. E.
1932. POTASH REQUIREMENTS OF THE TOBACCO CROP. Conn. (State) Agr. Expt. Sta. Bul. 334, 217 pp.

(2) Harris, R. G., Haley, D. E., and Reid, J. J.
1939. THE EFFECT OF FERTILIZER TREATMENT UPON THE TYPE OF FLORA FOUND UPON DECOMPOSING PLANT TISSUES. Soil Sci. Soc. Amer. Proc. (1938) 3: 183–186.

(3) James, W. O., and Penston, N. L.
1933. STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS. IV. THE QUANTITATIVE DISTRIBUTION OF POTASSIUM IN THE POTATO PLANT. Ann. Bot. [London] 47: [279]–293, illus.

(4) Janssen, George, and Bartholomew, R. P.
1929. THE TRANSLOCATION OF POTASSIUM IN TOMATO PLANTS AND ITS RELATION TO THEIR CARBOHYDRATE AND NITROGEN DISTRIBUTION. Jour. Agr. Res. 38: 447–465, illus.

(5) Janssen, George, and Bartholomew, R. P.
1930. THE INFLUENCE OF THE POTASH CONCENTRATION OF THE CULTURE MEDIUM ON THE PRODUCTION OF CARBOHYDRATES IN PLANTS. Jour. Agr. Res. 40: 243–261, illus.

(6) Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R.
1930. SOME EFFECTS OF POTASSIUM DEFICIENCY ON THE HISTOLOGICAL STRUCTURE AND NITROGENOUS AND CARBOHYDRATE CONSTITUENTS OF PLANTS. N. J. Agr. Expt. Sta. Bul. 499, 36 pp., illus.

(7) Quisumbing, F. A., and Thomas, A. W.
1921. CONDITIONS AFFECTING THE QUANTITATIVE DETERMINATION OF REDUCING SUGARS BY FEHLING SOLUTION. ELIMINATION OF CERTAIN ERRORS INVOLVED IN CURRENT METHODS. Amer. Chem. Soc. Jour. 43: 1503–1526, illus.

(8) Rohde, G.
1936. DIE WIRKUNG DES KALIUMS BEI DER KOHLENSÄUREASSIMILATION DER PFLANZEN. Ztschr. f. Pflanzenernähr., Düngung u. Bodenk. 44: [1]–24. [Abstract in Hoffer, G. N., Potash in Plant Metabolism, Indus. and Engin. Chem. 30: 887. 1938.]

(9) Schweizer, J.
1939. JAARVERSLAG TABAK OVER JULI 1938 T/M JUNI 1939. Besoek. Proefsta. [Java], Meded. 64, 64 pp. [Abstract in Better Crops With Plant Food 24 (3): 36. 1940.]

(10) Thomas, J. J., Haley, D. E., and Olson, Otto.
1936. THE ABSORPTION OF PLANT NUTRIENTS IN RELATION TO THE PRODUCTION OF PENNSYLVANIA CIGAR-LEAF TOBACCO. Pa. Agr. Expt. Sta. Bul. 331, 20 pp., illus.

○

Reprinted from JOURNAL OF DAIRY SCIENCE, July, 1941, Vol. XXIV, No. 7, pages 567–577.

UTILIZATION AND EXCRETION OF ASCORBIC ACID BY THE DAIRY COW*

C. A. KNIGHT, R. A. DUTCHER, N. B. GUERRANT
Department of Agricultural and Biological Chemistry
AND
S. I. BECHDEL
Department of Dairy Husbandry, The Pennsylvania State College, State College, Pennsylvania

The results of early studies (1, 2, 3) concerning the influence of diet upon the antiscorbutic potency of cow's milk, appeared to indicate that the vitamin C content of the milk paralleled that of the ration. During the same period, however, findings were reported (4) which led to the opposite conclusion, namely, that the ration received by cows had no influence on the antiscorbutic potency of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, further differences of opinion have arisen concerning the factors which influence the vitamin C content of cow's milk. As a result of recent work (5, 6, 7) the influence of breed and stage of lactation have been emphasized. Several workers (5, 8, 9) have attributed variations in the ascorbic acid content of milk to the season of the year. Other investigators (10, 11, 12, 13, 14) have concluded that the vitamin C content of cow's milk tends to be quite constant and is independent of the ration of the cow. Opposed to this view are those who still contend that the ascorbic acid content of the diet is a factor which cannot be ignored (6, 15).

A number of investigators have suggested that the cow is able to synthesize vitamin C (9, 13 ,16, 17, 18, 19). How or where this synthesis occurs is not understood, although one investigator (30) has claimed that the vitamin C of cow's milk is synthesized by the udder parenchyma and that the synthesis depends largely upon the condition of the udder.

The failure of numerous experiments involving standard dairy rations to establish clearly and conclusively the importance of the ascorbic acid content of the diet with regard to the amount of the vitamin in the milk, made it seem desirable to repeat previous work, but to alter the procedure by supplementing standard rations with known amounts of pure synthetic ascorbic acid. Moreover, it was desired to increase the significance of customary milk ascorbic acid analyses by determining, simultaneously, the amount of ascorbic acid in the blood and in 24-hour samples of urine.

Received for publication February 11, 1941.

* Authorized for publication on January 21, 1941, as paper No. 1016 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

If, in the above proposed studies, it could be shown conclusively that the vitamin C content of milk is independent of the ration of the cow, it was hoped that some explanation could be obtained for the anomalous results just mentioned. It was desired, for example, to find new evidence for the synthesis of ascorbic acid in the cow. Further, it was hoped that some clue might be found regarding both the metabolic fate of ascorbic acid and the factors which influence its elimination from the body in the milk and in the urine.

EXPERIMENTAL

Methods and Apparatus. One or both of two chemical methods of estimation were used in all ascorbic acid analyses. These were the Tillmans titration method (20) employing standard 2,6-dichlorobenzenoneindophenol, and the newer Roe furfural method (21), which estimates total ascorbic acid (both reduced and reversibly oxidized ascorbic acid).

Milk samples were taken in a special apparatus which has been shown to preserve all of the ascorbic acid of the milk in the reduced form (22). The use of this apparatus permitted the quantitative determination of the vitamin by the convenient indophenol titration method.

Blood samples were taken in the conventional oxalated tubes under paraffin oil. For each test, 20 to 30 ml. of blood were taken from the jugular vein of the cow. As soon as the sample was obtained, the collection apparatus was placed inside a dark glass receptacle containing ice and water and was taken immediately to the laboratory for analysis. Essentially the Farmer and Abt macro-method (23) for plasma ascorbic acid was used for routine blood analysis.

Successful collections of the total 24-hour urinary excretion, free from fecal contamination, were made by employing a special rubber urine tube developed by Forbes and coworkers (24). The 18-liter carboys, which were used as receivers in the collection apparatus, were painted black to exclude light and were charged with enough glacial acetic acid to give a final concentration of about 5 per cent by volume. Addition of stick metaphosphoric acid to the acetic acid appeared to give no better results than when acetic acid was used alone; consequently, the addition of metaphosphoric acid was discontinued. In urinary ascorbic acid analyses, both the indophenol titration and the Roe furfural method were employed, with the exception of the earliest work on one of the cows which was done before the furfural technique had been reported. This double analysis seemed desirable in view of the lack of unanimity among various workers concerning the specificity of present methods for the determination of ascorbic acid in urine. Moreover, it appeared probable that some of the vitamin would be unavoidably oxidized to dehydroascorbic acid during the collection of a 24-hour sample. Such has since been shown to be true with human urine (25). The furfural method

is particularly valuable in this case because it determines both dehydro- and reduced ascorbic acids.

It was recognized at the outset that it would be desirable to eliminate from the projected work the effect of breed differences by limiting the experiments to one breed of dairy cow. Holstein cows were eventually chosen, largely because they were available for experimental purposes at the time this work was started.

Effect of a Standard Ration. In order to have values which might later be used to compare with those obtained during administration of ascorbic acid, each cow was maintained on a standard ration for an interval of time during which analyses were made to determine the concentrations of ascorbic acid in the blood, the milk, and the urine. The averages of the values obtained during typical five-day test periods are given in table 1. Five-day sampling periods were considered representative for any diet, in view of the fact that all cows received a particular ration for several days, or even weeks, prior to the actual collection of samples.

TABLE 1

*Summary of results obtained when Holstein cows were fed a standard ration and when they were fed a standard ration supplemented with ascorbic acid**

	S. R.	C. S.	G. C.	D.
Mg. ascorbic acid per ml. milk	0.019	0.021	0.021	0.020
Mg. ascorbic acid per 100 ml. plasma	0.53	0.58	0.50	0.48
Mg. ascorbic acid per day in urine—				
Indophenol titration	45.7	52.7	54.1	26.8
Furfural method	910.5	833.1	643.5	

S. R.—Standard ration.

C. S.—Standard ration supplemented with 50 to 100 grams of ascorbic acid mixed with corn silage.

G. C.—Standard ration supplemented with 50 grams of ascorbic acid in gelatin capsules.

D.—Standard ration supplemented with 50 grams of ascorbic acid administered by drenching.

* The values given in Table 1 represent the averages obtained in experiments employing from two to four cows, except in the case of the ascorbic acid administered by drenching, where the values are from an experiment with a single cow.

Effect of Ascorbic Acid Added to Corn Silage. In choosing methods for administration of massive amounts of ascorbic acid, it was decided to administer some of the vitamin mixed with a small amount (3–5 pounds) of corn silage. It was found that 50–200 grams (1,000,000–4,000,000 International Units) of ascorbic acid placed in such a mixture were consumed by a cow in a period of about 10 minutes. In contrast, ascorbic acid mixed with grain was eaten very slowly, if at all.

Experimental periods were designed to include a two-day interval during which the cow received the standard ration alone, followed by a three-day period during which she received the standard ration supplemented each day with a certain amount of crystalline ascorbic acid mixed with corn silage, which was succeeded by a post-administrative period of two days during which the animal again received only the standard ration. The same procedure was used in subsequent experiments during which the ascorbic acid was administered in gelatin capsules and by drenching. The data from such experimental periods are summarized in table 1.

From a comparison of values in table 1, it is apparent that supplements of ascorbic acid administered by three different methods had no significant influence upon the concentrations of ascorbic acid in the blood, milk, or urine of the cows used in these experiments.

Effect of Ascorbic Acid Injected Intravenously. Rasmussen and coworkers (26) reported that intravenous injection of ascorbic acid resulted in a marked temporary rise in the vitamin C content of the milk of the ewe and cow. It was desired to repeat this work and to enlarge upon it by making analyses of blood and urine as well as of milk. Therefore, experiments were performed during which ascorbic acid was injected into the blood stream via the jugular vein. 24 grams of crystalline ascorbic acid, dissolved in sterile water, were given in this manner on each of two or three successive days. The effect of these ascorbic acid injections was studied with three cows with corresponding results. Typical values are given for one of the cows in table 2.

TABLE 2

Concentration of ascorbic acid in the blood, the milk, and in the urine of a Holstein cow during feeding of a standard ration supplemented with intravenous injections of ascorbic acid

Day	Mg. ascorbic acid per 100 ml. plasma	Mg. ascorbic acid per ml. milk	Mg. urinary ascorbic acid per day	
			Indophenol titration	Furfural method
1	0.58	0.021	24.3	611.1
2	0.58	0.020	34.6	241.5
3*	2.74**	0.020 0.019 0.022	6922.9	13311.8
4*	4.42**	0.024 0.028 0.028	9870.0	13632.6
5*	4.86**	0.030 0.029 0.027	14715.2	18194.0
6	0.86	0.028 0.025 0.022	8813.6	20923.8
7		0.021	161.2	604.7
8		0.020	117.8	982.8

* 24 grams of ascorbic acid dissolved in 100 ml. of sterile water were injected into the jugular vein. The multiple values for milk ascorbic acid represent each of the three daily milking periods.

** Blood analyses were made on samples taken 1½ hours after injection of ascorbic acid.

The intravenous injection of ascorbic acid produced unmistakable increases in the concentration of that vitamin in the milk and urine as well as in the blood. A very large portion of the injected ascorbic acid which could be accounted for appeared in the urine. In the case of one cow, this amounted to over ninety per cent of the total ascorbic acid injected. These results correspond closely to those recently reported (31) for experiments in which ascorbic acid was injected into goats.

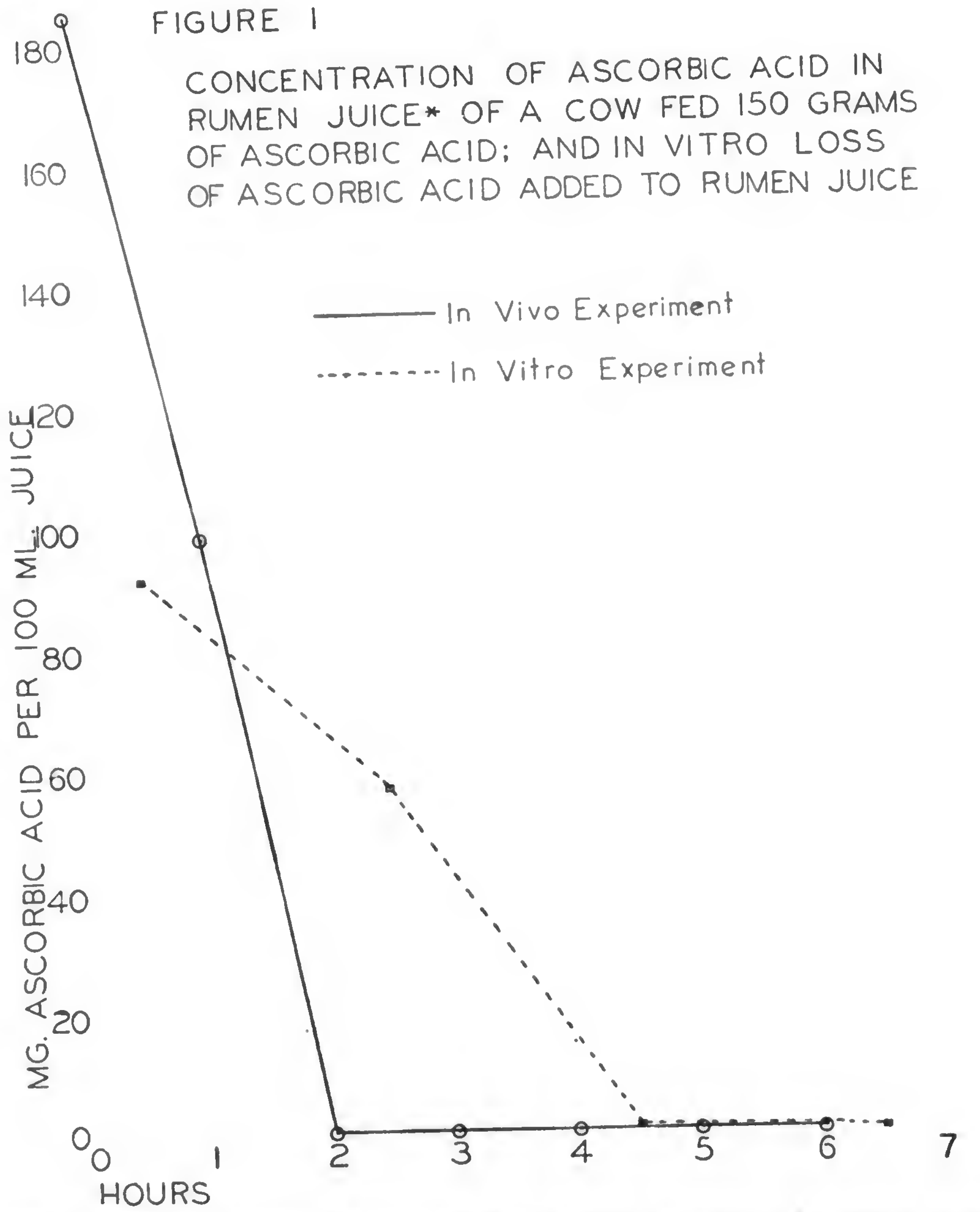
Effect of Ascorbic Acid Injected Subcutaneously. The experiments described thus far seemed to indicate that ascorbic acid administered by non-injection methods never reached the blood stream in significant amounts. Further, data from the intravenous injection experiments showed that the concentration of ascorbic acid in the milk was definitely increased if large amounts of the vitamin reached the blood stream. In order to further substantiate these findings, it was desired to administer some ascorbic acid by a method which would not place the vitamin directly in the blood stream but which would insure the ultimate arrival of large amounts in the circulation. Such a purpose was accomplished by setting up an experiment in which ascorbic acid was injected subcutaneously in regions around the cow's forelegs.

The effect of the subcutaneous injections of ascorbic acid closely resembled that of the intravenous injections. The influence of the subcutaneous injections upon blood, milk, and urinary ascorbic acid values was more gradual and somewhat less pronounced than in the case of the intravenous injections. During the course of three successive daily injections of 24 grams of ascorbic acid, the ascorbic acid titer of the milk was raised from 0.020 to 0.027 mg. per ml. and that of the urine from 600 to a peak of 15,000 mg. per day.

Studies with a Rumen Fistula. At this point in the experiments it appeared to be almost certain that the failure of massive amounts of ingested ascorbic acid to influence the concentration of the vitamin in the milk or to significantly alter its concentration in the blood and urine, could be attributed to a destruction of this substance in the rumen. To investigate this possibility, a rumen fistula was created in one of the cows. With this permanent opening leading directly into the largest compartment of the cow's stomach, it was possible to study the fate of ingested ascorbic acid by removal and analysis of partially digested food at intervals after feeding.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after feeding supplements of ascorbic acid and after insertion of the vitamin directly into the rumen. During such periods, the average concentrations of ascorbic acid in the blood plasma, in the milk, and in the urine were respectively 0.42 mg. per 100 ml., 0.019 mg. per ml., and 1260.5 mg. per day, values which do not differ significantly from those obtained during feeding of a standard ration alone.

The disappearance of ascorbic acid from the rumen contents during an experiment in which 150 grams of ascorbic acid were fed is shown graphically in figure 1. A preliminary report on this experiment has been given elsewhere (32).



* By the term "rumen juice" is meant the liquid portion of rumen contents.

In order to demonstrate that volume changes were not responsible for the pronounced decrease in the ascorbic acid concentration of the rumen contents as shown above, an experiment was performed in the laboratory with a controlled volume of rumen contents. 1000 ml. of rumen contents, from which the coarse particles of feed had been removed by straining through cheesecloth, were placed in a dark-glass, wide-mouth bottle. To this mixture, which had a pH of 6.50, was added 1000 mg. of crystalline ascorbic acid. After thoroughly mixing the contents, the bottle was loosely stoppered and placed in a water bath maintained at a temperature of 39° to 42° C. At intervals, samples were removed for analysis.

Figure 1 shows that the ascorbic acid disappeared just as it had in the *in vivo* experiments with the exception that the *in vitro* decrease proceeded at a more gradual rate. This slower rate of destruction of ascorbic acid in the *in vitro* experiment as compared to the *in vivo* experiments seems readily explained by the absence of the continual stirring and the circulation of gases so characteristic of the rumen.

DISCUSSION OF RESULTS

State of Ascorbic Acid in Urine. As soon as the furfural method was applied to the urine analyses it became apparent that the results obtained ranged from one and a half to thirty times as high as those obtained by the indophenol titration. During injections of ascorbic acid, however, this discrepancy narrowed to a point where, in some cases, the results obtained by the two methods almost coincided. These facts, together with the knowledge that the indophenol and furfural methods of analysis checked well when applied to 24-hour samples of rat urine collected under similar conditions (33), suggested that important amounts of the ascorbic acid excreted daily by a cow on a standard ration were excreted in the form of dehydroascorbic acid. In order to test this hypothesis, samples of urine were collected as they were excreted and analyzed at once. Table 3 gives the results obtained from Holstein, Jersey, Brown Swiss, and Ayrshire cows. These findings seem to indicate that all the ascorbic acid excreted in the urine of the dairy cow is in the reduced form. The greater values obtained in eight out of ten cases by the indophenol method are readily explained by the recognized tendency to go beyond the endpoint, which is obscured to an extent proportional to the concentration of the urine pigments. The greater values obtained by the furfural method for 24-hour samples of urine must indicate, therefore, that variable amounts of the excreted ascorbic acid are oxidized to dehydroascorbic acid during the interval between excretion and analysis, or that the urine contains appreciable amounts of non-ascorbic acid furfural precursors capable of forming a derivative with 2,4-dinitrophenylhydrazine. Tests for the latter interfering substances were made repeatedly and in no case showed the presence of concentrations sufficiently large to interfere with

TABLE 3

Reduced and oxidized ascorbic acid in cow's urine immediately following urination

Cow No.	Reduced ascorbic acid Mg. per liter Indophenol titration	Total ascorbic acid Mg. per liter Furfural method
1	20.9	17.0
2	33.2	25.4
3	19.7	18.2
4	10.1	10.3
5	27.7	25.1
6	27.6	25.4
7	53.1	48.1
8	40.1	43.3
9	21.5	19.0
10	31.0	29.6

the method. Consequently, it may be concluded that while all the ascorbic acid excreted in the urine is originally in the reduced form, some of it is oxidized to dehydroascorbic acid under the conditions involved in the collection of a 24-hour sample of urine.

Destruction of Ascorbic Acid in the Rumen. Several explanations might be given for the rapid and pronounced disappearance of ascorbic acid from the rumen after oral administration of massive amounts of the vitamin. It might be argued that the large amounts observed in the first two or three samples represented stages in the incomplete mixing of the ascorbic acid with rumen contents. Changes in the concentration of the rumen ascorbic acid could also be attributed to an intake of water by the animal, or by a passage of a portion of the rumen contents to other chambers of the stomach.

Another interpretation of the findings is that the very soluble vitamin was rapidly absorbed. This has been suggested by Riddell and Whitnah (27). These workers studied the fate of vitamin C in the rumen contents of a cow with a rumen fistula and in a steer at slaughter. In each case the rumen contents were found to contain less than one-tenth the vitamin C of green rye ingested twelve hours earlier. These workers explained the rapid disappearance of ascorbic acid from the rumen by suggesting that ascorbic acid was rapidly absorbed. The basis for this suggestion was the observation of a temporary doubling of the vitamin C content of the blood within 12 hours and a fivefold increase in the ascorbic acid content of the urine within 60 hours after green feed was first supplied.

The most plausible explanation, however, and the one demanded by our experimental data, is that a rapid and pronounced destruction of ingested ascorbic acid occurs in the rumen. Thus, while the other factors which have been mentioned undoubtedly have some influence on the results observed, it is hardly likely that they account for changes of the magnitude indicated in figure 1.

The incomplete mixing theory is especially inadequate. When a cow is eating, the rumen contracts about three times per minute and each contraction causes a flow of liquid throughout the rumen and its solid contents. While figure 1 indicates that the first sample was removed at zero time, it was actually taken about five minutes after the animal had eaten the last of the ascorbic acid treated silage or 15 minutes after she had started to eat. This means that the ascorbic acid had been subjected to 15 to 45 contractions of the rumen. Further, when the cow was slaughtered, it was found that the rumen contained 38 liters of liquid or semi-liquid material. If 150 grams of ascorbic acid were thoroughly distributed throughout this liquid, there should be a concentration equivalent to about 400 mg. per 100 ml. of juice. Figure 1 indicates that the concentration of ascorbic acid in the juice, as shown by analysis of the first sample removed, was about 185 mg. per 100 ml. of juice. From these facts, it would appear that fairly complete mixing had occurred, accompanied by extensive destruction of the vitamin.

Observations of this and other dairy cows show that they drink water infrequently. It is difficult to say how much and how often liquid material passes permanently from the rumen into other compartments of the stomach. Ewing and Wright (28), working with steers, found that the average rate of passage of food residues through the rumen and reticulum was 61 hours. In any event, credence in the volume-change explanation for the disappearance of ascorbic acid from the rumen is seriously discounted by the results of *in vitro* constant-volume experiments such as shown in figure 1.

Indirect evidence that ingested ascorbic acid is largely destroyed in the rumen rather than being rapidly absorbed is given in the blood, milk, and urine ascorbic acid values, which, with the possible exception of the urine, show no response to the feeding of 50 grams or more of ascorbic acid. Why there is always a small amount of ascorbic acid in the rumen, as there appears to be, is difficult to explain. Possibly the release of this vitamin from solid feed fragments proceeds gradually and at a rate slightly higher than the rate of destruction. We hope to continue the study of the factors responsible for the destructive effects described.

In the light of the demonstrated destruction of ascorbic acid in the rumen, it becomes clear why various methods of oral administration of the vitamin failed to produce a response in the milk or other body fluids. If the ascorbic acid administered in gelatin capsules had been able to survive rumen conditions and reach other compartments of the stomach, it seems likely that other results might have been obtained, for the ascorbic acid content of the milk of non-ruminating animals, *e.g.*, guinea pigs (26) and humans (8, 29), has been shown to be influenced by diet. In work with the rumen fistula, however, it was found that gelatin capsules were dissolved and the ascorbic acid was released after 10–15 minutes in the rumen.

SUMMARY

1. Special equipment was employed which permitted the complete collection from dairy cows of 24-hour samples of urine free from any fecal contamination. It was found impractical, if not impossible, to preserve all the ascorbic acid in such urine samples in the reduced form.

2. By the simultaneous application of the indophenol titration and the furfural method of analysis to freshly excreted samples of urine, it was possible to show that ascorbic acid is excreted in cow's urine in the reduced form.

3. Analysis of over 50 samples of blood obtained from four Holstein cows showed that the ascorbic acid content of the plasma ranged from 0.43 to 0.62 mg. per 100 ml. when the cows received standard dairy rations.

4. Ascorbic acid was administered to Holstein cows (a) mixed with a small amount of corn silage, (b) in gelatin capsules, (c) in aqueous solution, (d) intravenous injection, and (e) by subcutaneous injection. Administration of as high as 100 grams (2,000,000 International Units) of ascorbic acid per day for three days by a non-injection method failed to increase the ascor-

bic acid concentration of the milk or blood and had only a slight effect on the concentration of the vitamin in the urine. It was only by the injection methods that a significant increase in the ascorbic acid concentrations of the blood, milk, and urine could be demonstrated. The greatest increase in milk ascorbic acid concentration during experiments in which 24 grams of ascorbic acid were injected intravenously on each of three successive days, was from 20 mg. per liter to 30 mg. per liter.

5. A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed as much as 150 grams (3,000,000 International Units) of synthetic ascorbic acid at one time; similar amounts were also placed directly in the rumen through the fistula opening. A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals. Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered receptacle at 39°–42° C. disappeared at much the same rate as that of the *in vivo* experiments. In making analyses, both the indophenol titration and the Roe furfural method were employed.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

REFERENCES

(1) Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mead, S. W., and Schaefer, O. G. Vitamin studies VI. The influence of diet of the cow upon the nutritive and antiscorbutic properties of cow's milk. J. Biol. Chem., **45**: 119–132. 1920.

(2) Hart, E. B., Steenbock, H., and Ellis, N. R. Influence of diet on the antiscorbutic potency of milk. J. Biol. Chem., **42**: 383–396. 1920.

(3) Hess, A. F., Unger, L. J., and Supplee, G. C. Relation of fodder to the antiscorbutic potency and salt content of milk. J. Biol. Chem., **45**: 229–235. 1920.

(4) Hughes, J. S., Fitch, J. B., and Cave, H. W. The quantitative relation between the vitamin content of feed eaten and milk produced. J. Biol. Chem., **46**: p. L. 1921.

(5) Holmes, A. D., Tripp, F., Woelffer, E. A., and Satterfield, G. H. A study of breed and seasonal variations in the ascorbic acid content of certified milk from Guernseys and Holsteins. J. Nutrit., **17**: 187–198. 1939.

(6) Rasmussen, R., Guerrant, N. B., Shaw, A. O., Welch, R. C., and Bechdel, S. I. The effects of breed characteristics and stages of lactation on the vitamin C (ascorbic acid) content of cow's milk. J. Nutrit., **11**: 425–432. 1936.

(7) Whitnah, C. H., and Riddell, W. H. Variations in the vitamin C content of milk. J. Dairy Sc., **20**: 9–14. 1937.

(8) Ferdinand, H. Vitamin C content of human and cow's milk in spring. Klin. Wochschr., **15**: 1311–1312. 1936.

(9) Stoerr, E. Milk as a source of vitamin C. Human milk; cow's milk raw and heated; seasonal variations; condensed and dried milk. Rev. franc. Pediat., **12**: 427. 1936.

(10) Cultrera, R., and Bellini, B. Vitamin C of milk in relation to period of lactation and feeding. Ann. Chim. appl. Roma, **28**: 217. 1938.

(11) Harris, L. J., and Ray, S. N. Diagnosis of vitamin C subnutrition by urine analysis with a note on the antiscorbutic value of human milk. Lancet, **228**: 71–77. 1935.

(12) Jacobsen, J. E. Cause of the low stability of vitamin C in milk. Ztschr. für Untersuch. der Lebensmittel, **69**: 306–313. 1935.

(13) Kon, S. K., and Watson, M. B. The vitamin C content of cow's milk. Biochem. J., **31**: 223–226. 1937.

(14) Riddell, W. H., Whitnah, C. H., Hughes, J. S., and Lienhardt, H. F. Influence of the ration on the vitamin C content of milk. J. Nutrit., **11**: 47–54. 1936.

(15) Neuweiler, W. Vitamin C content of human milk. Z. Vitaminforsch., **4**: 39. 1935.

(16) Hughes, J. S., Fitch, J. B., Cave, H. W., and Riddell, W. H. Relation between the vitamin C content of a cow's ration and the vitamin C content of its milk. J. Biol. Chem., **71**: 309–316. 1927.

(17) Reedman, E. J. The ascorbic acid content of milk. Canada Public Health Jour., **28**: 339–340. 1937.

(18) Riddell, W. H., Whitnah, C. H., and Hughes, J. S. Influence of the ration on the vitamin C of milk. J. Dairy Sc., **18**: 437. 1935.

(19) Thurston, L. M., Palmer, L. S., and Eckles, C. H. Further studies of the role of vitamin C in the nutrition of calves. J. Dairy Sc., **12**: 394–404. 1929.

(20) Tillmans, J. The antiscorbutic vitamin. Ztschr. für Untersuch. der Lebensmittel, **60**: 34–44. 1930.

(21) Roe, J. H., and Hall, J. M. The vitamin C content of human urine and its determination through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., **128**: 329–337. 1939.

(22) Knight, C. A., Dutcher, R. A., and Guerrant, N. B. The quantitative determination of vitamin C in milk. Science, **89**: 183. 1939.

(23) Farmer, C. J., and Abt, A. F. Determination of reduced ascorbic acid in small amounts of blood. Proc. Soc. Exptl. Biol. Med., **34**: 146. 1936.

(24) Forbes, E. B., Bratzler, J. W., Black, A., and Braman, W. W. The digestibility of rations by cattle and sheep. Penna. Agr. Exp. Sta. Bull. 339. 1937.

(25) Holmes, H. N., and Campbell, K. The determination of vitamin C in urine. Jour. Lab. Clin. Med., **24**: 1293–1296. 1939.

(26) Rasmussen, R., Bogart, R., and Maynard, L. A. Ascorbic acid content of milk of various species as influenced by ascorbic acid injection and diet. Proc. Soc. Exptl. Biol. Med., **39**: 502. 1938.

(27) Riddell, W. H., and Whitnah, C. H. Vitamin C metabolism in the dairy cow (Abstract). J. Dairy Sc., **21**: 121. 1938.

(28) Ewing, P. V., and Wright, L. H. A study of the physical changes in feed residues which take place in cattle during digestion. J. Agr. Res., **13**: 639–646. 1918.

(29) Selleg, I., and King, C. G. The vitamin C content of human milk and its variation with diet. J. Nutrit., **11**: 599–606. 1936.

(30) Wendt, G. V. Vitamin C studies. The vitamin C content of cow milk. Skand. Arch. Physiol., **80**: 398–402. 1938.

(31) Richmond, M. S., Satterfield, G. H., Grinnells, C. D., and Dann, W. J. Ascorbic acid content of goat's milk and blood: Influence of ascorbic acid injection and diet. J. Nutrit., **20**: 99. 1940.

(32) Knight, C. A., Dutcher, R. A., Guerrant, N. B., and Bechdel, S. I. Destruction of ascorbic acid in the rumen of the dairy cow. Proc. Soc. Exptl. Biol. Med., **44**: 90. 1940.

(33) Knight, C. A. Unpublished data.

The disappearance of ascorbic acid from the rumen contents during an experiment in which 150 grams of ascorbic acid were fed is shown graphically in figure 1. A preliminary report on this experiment has been given elsewhere (32).

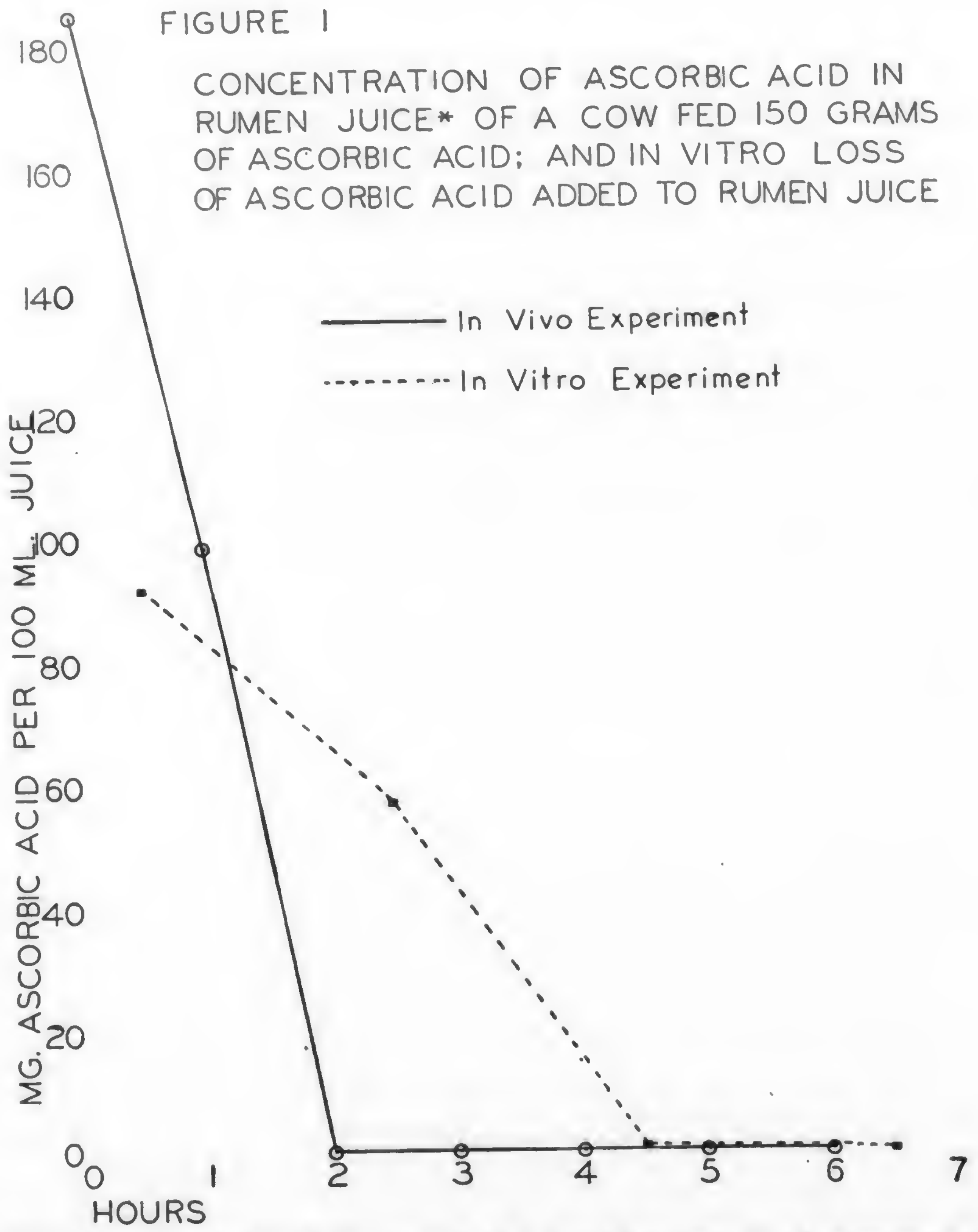


FIGURE 1

CONCENTRATION OF ASCORBIC ACID IN RUMEN JUICE* OF A COW FED 150 GRAMS OF ASCORBIC ACID; AND IN VITRO LOSS OF ASCORBIC ACID ADDED TO RUMEN JUICE

* By the term "rumen juice" is meant the liquid portion of rumen contents.

In order to demonstrate that volume changes were not responsible for the pronounced decrease in the ascorbic acid concentration of the rumen contents as shown above, an experiment was performed in the laboratory with a controlled volume of rumen contents. 1000 ml. of rumen contents, from which the coarse particles of feed had been removed by straining through cheesecloth, were placed in a dark-glass, wide-mouth bottle. To this mixture, which had a pH of 6.50, was added 1000 mg. of crystalline ascorbic acid. After thoroughly mixing the contents, the bottle was loosely stoppered and placed in a water bath maintained at a temperature of 39° to 42° C. At intervals, samples were removed for analysis.

Figure 1 shows that the ascorbic acid disappeared just as it had in the *in vivo* experiments with the exception that the *in vitro* decrease proceeded at a more gradual rate. This slower rate of destruction of ascorbic acid in the *in vitro* experiment as compared to the *in vivo* experiments seems readily explained by the absence of the continual stirring and the circulation of gases so characteristic of the rumen.

DISCUSSION OF RESULTS

State of Ascorbic Acid in Urine. As soon as the furfural method was applied to the urine analyses it became apparent that the results obtained ranged from one and a half to thirty times as high as those obtained by the indophenol titration. During injections of ascorbic acid, however, this discrepancy narrowed to a point where, in some cases, the results obtained by the two methods almost coincided. These facts, together with the knowledge that the indophenol and furfural methods of analysis checked well when applied to 24-hour samples of rat urine collected under similar conditions (33), suggested that important amounts of the ascorbic acid excreted daily by a cow on a standard ration were excreted in the form of dehydroascorbic acid. In order to test this hypothesis, samples of urine were collected as they were excreted and analyzed at once. Table 3 gives the results obtained from Holstein, Jersey, Brown Swiss, and Ayrshire cows. These findings seem to indicate that all the ascorbic acid excreted in the urine of the dairy cow is in the reduced form. The greater values obtained in eight out of ten cases by the indophenol method are readily explained by the recognized tendency to go beyond the endpoint, which is obscured to an extent proportional to the concentration of the urine pigments. The greater values obtained by the furfural method for 24-hour samples of urine must indicate, therefore, that variable amounts of the excreted ascorbic acid are oxidized to dehydroascorbic acid during the interval between excretion and analysis, or that the urine contains appreciable amounts of non-ascorbic acid furfural precursors capable of forming a derivative with 2,4-dinitrophenylhydrazine. Tests for the latter interfering substances were made repeatedly and in no case showed the presence of concentrations sufficiently large to interfere with

TABLE 3

Reduced and oxidized ascorbic acid in cow's urine immediately following urination

Cow No.	Reduced ascorbic acid Mg. per liter Indophenol titration	Total ascorbic acid Mg. per liter Furfural method
1	20.9	17.0
2	33.2	25.4
3	19.7	18.2
4	10.1	10.3
5	27.7	25.1
6	27.6	25.4
7	53.1	48.1
8	40.1	43.3
9	21.5	19.0
10	31.0	29.6

the method. Consequently, it may be concluded that while all the ascorbic acid excreted in the urine is originally in the reduced form, some of it is oxidized to dehydroascorbic acid under the conditions involved in the collection of a 24-hour sample of urine.

Destruction of Ascorbic Acid in the Rumen. Several explanations might be given for the rapid and pronounced disappearance of ascorbic acid from the rumen after oral administration of massive amounts of the vitamin. It might be argued that the large amounts observed in the first two or three samples represented stages in the incomplete mixing of the ascorbic acid with rumen contents. Changes in the concentration of the rumen ascorbic acid could also be attributed to an intake of water by the animal, or by a passage of a portion of the rumen contents to other chambers of the stomach.

Another interpretation of the findings is that the very soluble vitamin was rapidly absorbed. This has been suggested by Riddell and Whitnah (27). These workers studied the fate of vitamin C in the rumen contents of a cow with a rumen fistula and in a steer at slaughter. In each case the rumen contents were found to contain less than one-tenth the vitamin C of green rye ingested twelve hours earlier. These workers explained the rapid disappearance of ascorbic acid from the rumen by suggesting that ascorbic acid was rapidly absorbed. The basis for this suggestion was the observation of a temporary doubling of the vitamin C content of the blood within 12 hours and a fivefold increase in the ascorbic acid content of the urine within 60 hours after green feed was first supplied.

The most plausible explanation, however, and the one demanded by our experimental data, is that a rapid and pronounced destruction of ingested ascorbic acid occurs in the rumen. Thus, while the other factors which have been mentioned undoubtedly have some influence on the results observed, it is hardly likely that they account for changes of the magnitude indicated in figure 1.

The incomplete mixing theory is especially inadequate. When a cow is eating, the rumen contracts about three times per minute and each contraction causes a flow of liquid throughout the rumen and its solid contents. While figure 1 indicates that the first sample was removed at zero time, it was actually taken about five minutes after the animal had eaten the last of the ascorbic acid treated silage or 15 minutes after she had started to eat. This means that the ascorbic acid had been subjected to 15 to 45 contractions of the rumen. Further, when the cow was slaughtered, it was found that the rumen contained 38 liters of liquid or semi-liquid material. If 150 grams of ascorbic acid were thoroughly distributed throughout this liquid, there should be a concentration equivalent to about 400 mg. per 100 ml. of juice. Figure 1 indicates that the concentration of ascorbic acid in the juice, as shown by analysis of the first sample removed, was about 185 mg. per 100 ml. of juice. From these facts, it would appear that fairly complete mixing had occurred, accompanied by extensive destruction of the vitamin.

Observations of this and other dairy cows show that they drink water infrequently. It is difficult to say how much and how often liquid material passes permanently from the rumen into other compartments of the stomach. Ewing and Wright (28), working with steers, found that the average rate of passage of food residues through the rumen and reticulum was 61 hours. In any event, credence in the volume-change explanation for the disappearance of ascorbic acid from the rumen is seriously discounted by the results of *in vitro* constant-volume experiments such as shown in figure 1.

Indirect evidence that ingested ascorbic acid is largely destroyed in the rumen rather than being rapidly absorbed is given in the blood, milk, and urine ascorbic acid values, which, with the possible exception of the urine, show no response to the feeding of 50 grams or more of ascorbic acid. Why there is always a small amount of ascorbic acid in the rumen, as there appears to be, is difficult to explain. Possibly the release of this vitamin from solid feed fragments proceeds gradually and at a rate slightly higher than the rate of destruction. We hope to continue the study of the factors responsible for the destructive effects described.

In the light of the demonstrated destruction of ascorbic acid in the rumen, it becomes clear why various methods of oral administration of the vitamin failed to produce a response in the milk or other body fluids. If the ascorbic acid administered in gelatin capsules had been able to survive rumen conditions and reach other compartments of the stomach, it seems likely that other results might have been obtained, for the ascorbic acid content of the milk of non-ruminating animals, *e.g.*, guinea pigs (26) and humans (8, 29), has been shown to be influenced by diet. In work with the rumen fistula, however, it was found that gelatin capsules were dissolved and the ascorbic acid was released after 10–15 minutes in the rumen.

SUMMARY

1. Special equipment was employed which permitted the complete collection from dairy cows of 24-hour samples of urine free from any fecal contamination. It was found impractical, if not impossible, to preserve all the ascorbic acid in such urine samples in the reduced form.

2. By the simultaneous application of the indophenol titration and the furfural method of analysis to freshly excreted samples of urine, it was possible to show that ascorbic acid is excreted in cow's urine in the reduced form.

3. Analysis of over 50 samples of blood obtained from four Holstein cows showed that the ascorbic acid content of the plasma ranged from 0.43 to 0.62 mg. per 100 ml. when the cows received standard dairy rations.

4. Ascorbic acid was administered to Holstein cows (a) mixed with a small amount of corn silage, (b) in gelatin capsules, (c) in aqueous solution, (d) intravenous injection, and (e) by subcutaneous injection. Administration of as high as 100 grams (2,000,000 International Units) of ascorbic acid per day for three days by a non-injection method failed to increase the ascor-

bic acid concentration of the milk or blood and had only a slight effect on the concentration of the vitamin in the urine. It was only by the injection methods that a significant increase in the ascorbic acid concentrations of the blood, milk, and urine could be demonstrated. The greatest increase in milk ascorbic acid concentration during experiments in which 24 grams of ascorbic acid were injected intravenously on each of three successive days, was from 20 mg. per liter to 30 mg. per liter.

5. A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed as much as 150 grams (3,000,000 International Units) of synthetic ascorbic acid at one time; similar amounts were also placed directly in the rumen through the fistula opening. A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals. Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered receptacle at 39°–42° C. disappeared at much the same rate as that of the *in vivo* experiments. In making analyses, both the indophenol titration and the Roe furfural method were employed.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

REFERENCES

(1) Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mead, S. W., and Schaefer, O. G. Vitamin studies VI. The influence of diet of the cow upon the nutritive and antiscorbutic properties of cow's milk. J. Biol. Chem., **45**: 119–132. 1920.

(2) Hart, E. B., Steenbock, H., and Ellis, N. R. Influence of diet on the antiscorbutic potency of milk. J. Biol. Chem., **42**: 383–396. 1920.

(3) Hess, A. F., Unger, L. J., and Supplee, G. C. Relation of fodder to the antiscorbutic potency and salt content of milk. J. Biol. Chem., **45**: 229–235. 1920.

(4) Hughes, J. S., Fitch, J. B., and Cave, H. W. The quantitative relation between the vitamin content of feed eaten and milk produced. J. Biol. Chem., **46**: p. L. 1921.

(5) Holmes, A. D., Tripp, F., Woelffer, E. A., and Satterfield, G. H. A study of breed and seasonal variations in the ascorbic acid content of certified milk from Guernseys and Holsteins. J. Nutrit., **17**: 187–198. 1939.

(6) Rasmussen, R., Guerrant, N. B., Shaw, A. O., Welch, R. C., and Bechdel, S. I. The effects of breed characteristics and stages of lactation on the vitamin C (ascorbic acid) content of cow's milk. J. Nutrit., **11**: 425–432. 1936.

(7) Whitnah, C. H., and Riddell, W. H. Variations in the vitamin C content of milk. J. Dairy Sc., **20**: 9–14. 1937.

(8) Ferdinand, H. Vitamin C content of human and cow's milk in spring. Klin. Wochschr., **15**: 1311–1312. 1936.

(9) Stoerr, E. Milk as a source of vitamin C. Human milk; cow's milk raw and heated; seasonal variations; condensed and dried milk. Rev. franc. Pediat., **12**: 427. 1936.

(10) Cultrera, R., and Bellini, B. Vitamin C of milk in relation to period of lactation and feeding. Ann. Chim. appl. Roma, **28**: 217. 1938.

(11) Harris, L. J., and Ray, S. N. Diagnosis of vitamin C subnutrition by urine analysis with a note on the antiscorbutic value of human milk. Lancet, **228**: 71–77. 1935.

(12) Jacobsen, J. E. Cause of the low stability of vitamin C in milk. Ztschr. für Untersuch. der Lebensmittel, **69**: 306–313. 1935.

(13) Kon, S. K., and Watson, M. B. The vitamin C content of cow's milk. Biochem. J., **31**: 223–226. 1937.

(14) Riddell, W. H., Whitnah, C. H., Hughes, J. S., and Lienhardt, H. F. Influence of the ration on the vitamin C content of milk. J. Nutrit., **11**: 47–54. 1936.

(15) Neuweiler, W. Vitamin C content of human milk. Z. Vitaminforsch., **4**: 39. 1935.

(16) Hughes, J. S., Fitch, J. B., Cave, H. W., and Riddell, W. H. Relation between the vitamin C content of a cow's ration and the vitamin C content of its milk. J. Biol. Chem., **71**: 309–316. 1927.

(17) Reedman, E. J. The ascorbic acid content of milk. Canada Public Health Jour., **28**: 339–340. 1937.

(18) Riddell, W. H., Whitnah, C. H., and Hughes, J. S. Influence of the ration on the vitamin C of milk. J. Dairy Sc., **18**: 437. 1935.

(19) Thurston, L. M., Palmer, L. S., and Eckles, C. H. Further studies of the role of vitamin C in the nutrition of calves. J. Dairy Sc., **12**: 394–404. 1929.

(20) Tillmans, J. The antiscorbutic vitamin. Ztschr. für Untersuch. der Lebensmittel, **60**: 34–44. 1930.

(21) Roe, J. H., and Hall, J. M. The vitamin C content of human urine and its determination through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., **128**: 329–337. 1939.

(22) Knight, C. A., Dutcher, R. A., and Guerrant, N. B. The quantitative determination of vitamin C in milk. Science, **89**: 183. 1939.

(23) Farmer, C. J., and Abt, A. F. Determination of reduced ascorbic acid in small amounts of blood. Proc. Soc. Exptl. Biol. Med., **34**: 146. 1936.

(24) Forbes, E. B., Bratzler, J. W., Black, A., and Braman, W. W. The digestibility of rations by cattle and sheep. Penna. Agr. Exp. Sta. Bull. 339. 1937.

(25) Holmes, H. N., and Campbell, K. The determination of vitamin C in urine. Jour. Lab. Clin. Med., **24**: 1293–1296. 1939.

(26) Rasmussen, R., Bogart, R., and Maynard, L. A. Ascorbic acid content of milk of various species as influenced by ascorbic acid injection and diet. Proc. Soc. Exptl. Biol. Med., **39**: 502. 1938.

(27) Riddell, W. H., and Whitnah, C. H. Vitamin C metabolism in the dairy cow (Abstract). J. Dairy Sc., **21**: 121. 1938.

(28) Ewing, P. V., and Wright, L. H. A study of the physical changes in feed residues which take place in cattle during digestion. J. Agr. Res., **13**: 639–646. 1918.

(29) Selleg, I., and King, C. G. The vitamin C content of human milk and its variation with diet. J. Nutrit., **11**: 599–606. 1936.

(30) Wendt, G. V. Vitamin C studies. The vitamin C content of cow milk. Skand. Arch. Physiol., **80**: 398–402. 1938.

(31) Richmond, M. S., Satterfield, G. H., Grinnells, C. D., and Dann, W. J. Ascorbic acid content of goat's milk and blood: Influence of ascorbic acid injection and diet. J. Nutrit., **20**: 99. 1940.

(32) Knight, C. A., Dutcher, R. A., Guerrant, N. B., and Bechdel, S. I. Destruction of ascorbic acid in the rumen of the dairy cow. Proc. Soc. Exptl. Biol. Med., **44**: 90. 1940.

(33) Knight, C. A. Unpublished data.

Reprinted from Analytica
INDUSTRIAL AND ENGINEER
Vol. 13, Page 512, July

New Science Building
State Coll

R. ADAMS DUTCHER, Pennsylvania State

THE Departments of Agricultural and Biological Chemistry and Zoology and Economic Entomology of Pennsylvania State College are now occupying the new Agricultural Science Building, one of the several new buildings made available by the General State Authority in 1940. This brief description deals solely with the facilities of the Department of Agricultural and Biological Chemistry.

The building has been named Frear Laboratories in honor of the late William Frear, nationally known agricultural chemist, who devoted 37 years to Pennsylvania agriculture and national pure food problems. It is constructed of cream-colored Roman brick, trimmed with Indiana limestone.

The
floors
above
groun

All
tile
brack
have
ing t
throu
venie

Flo

of black linoleum
wood construction
laminated pressed
plastic finish. De
with gas, cold and
lines, steam, and

Reprinted from Analytical Edition
INDUSTRIAL AND ENGINEERING CHEMISTRY
Vol. 13, Page 512, July 15, 1941

New Science Building at Pennsylvania State College

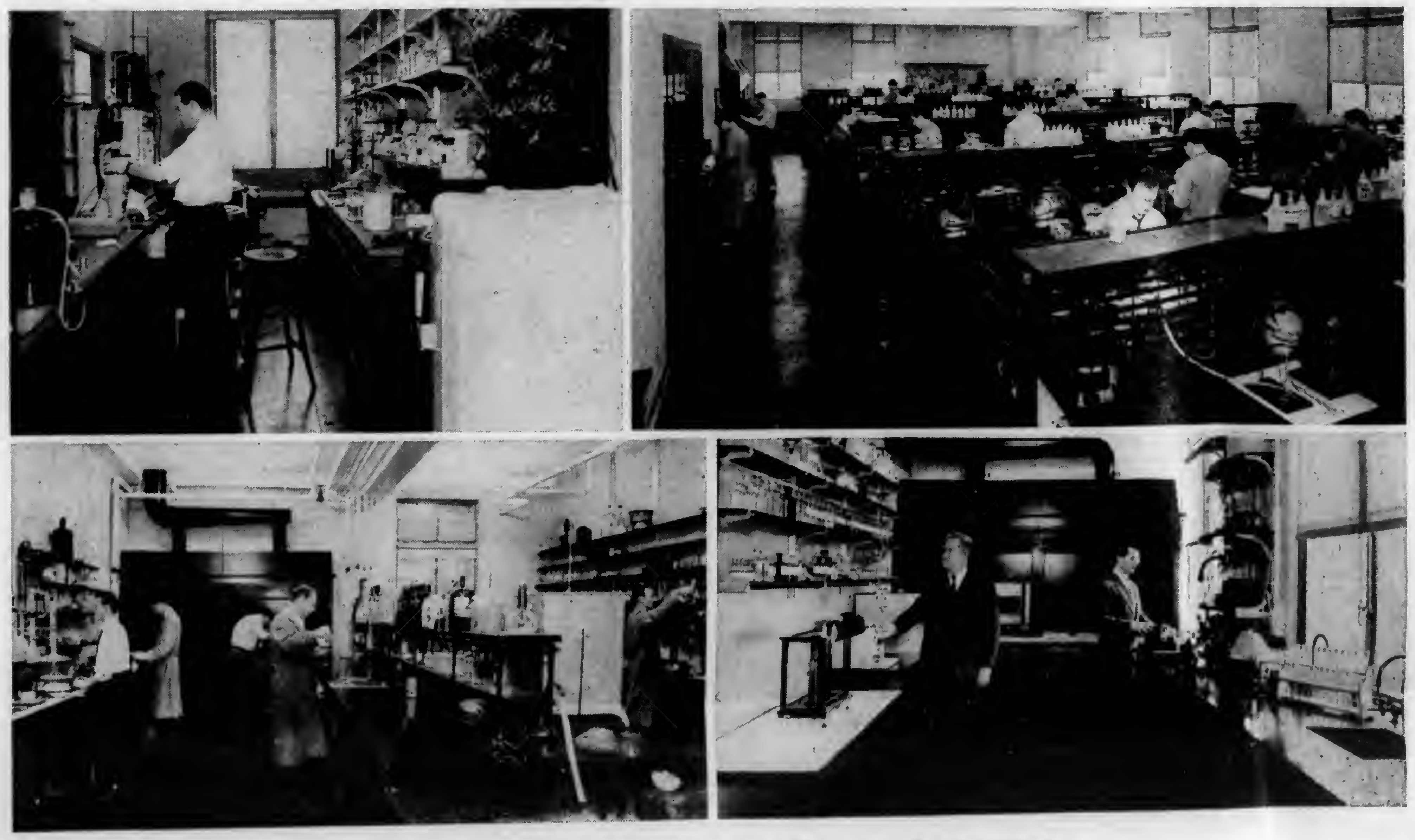
R. ADAMS DUTCHER, Pennsylvania State College, State College, Penna.

THE Departments of Agricultural and Biological Chemistry and Zoology and Economic Entomology of Pennsylvania State College are now occupying the new Agricultural Science Building, one of the several new buildings made available by the General State Authority in 1940. This brief description deals solely with the facilities of the Department of Agricultural and Biological Chemistry.

The building has been named Frear Laboratories in honor of the late William Frear, nationally known agricultural chemist, who devoted 37 years to Pennsylvania agriculture and national pure food problems. It is constructed of cream-colored Roman brick, trimmed with Indiana limestone. The building measures 205 by 70 feet and consists of four floors. Owing to the contour of the campus three floors are above ground level on the front, while four floors are above ground at the rear.

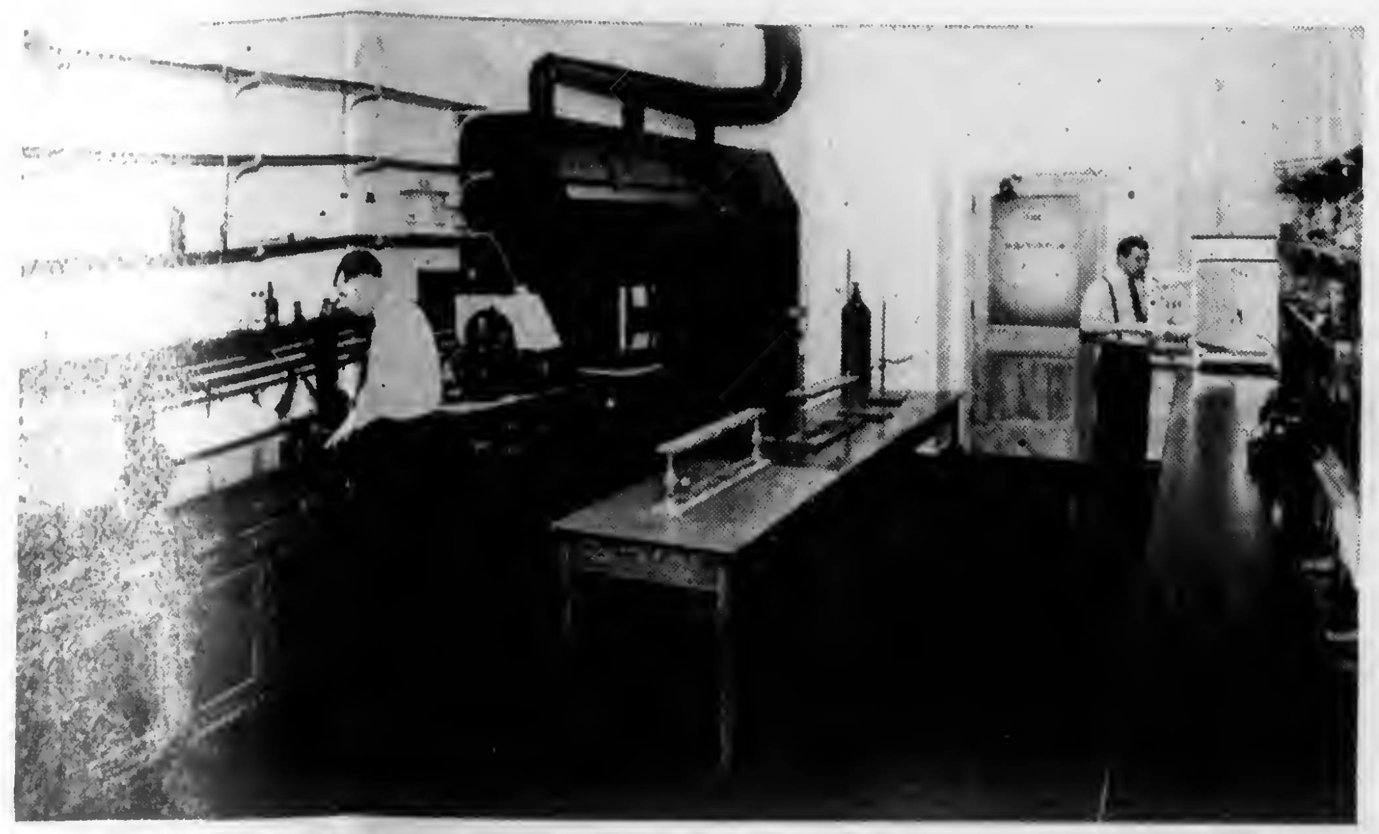
All laboratories are well lighted and have cream-colored tile walls equipped with adjustable shelving on aluminum brackets set in aluminum slots. This makes it possible to have shelves of any desired width or height without mutilating the walls. Acidproof plumbing is standard equipment throughout and all plumbing is exposed (overhead) for convenience in making repairs.

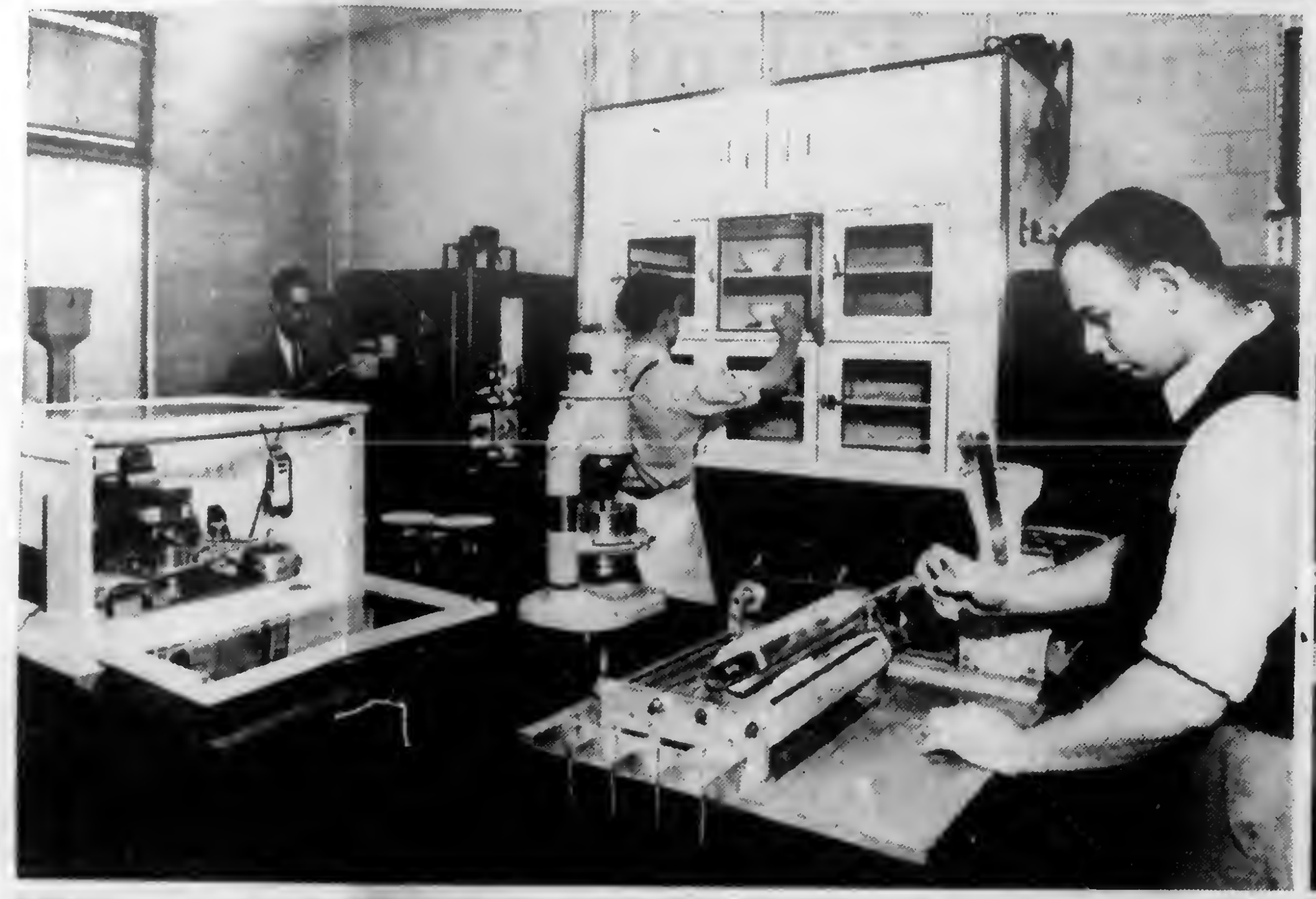
Floors, with the exception of a few special rooms, consist



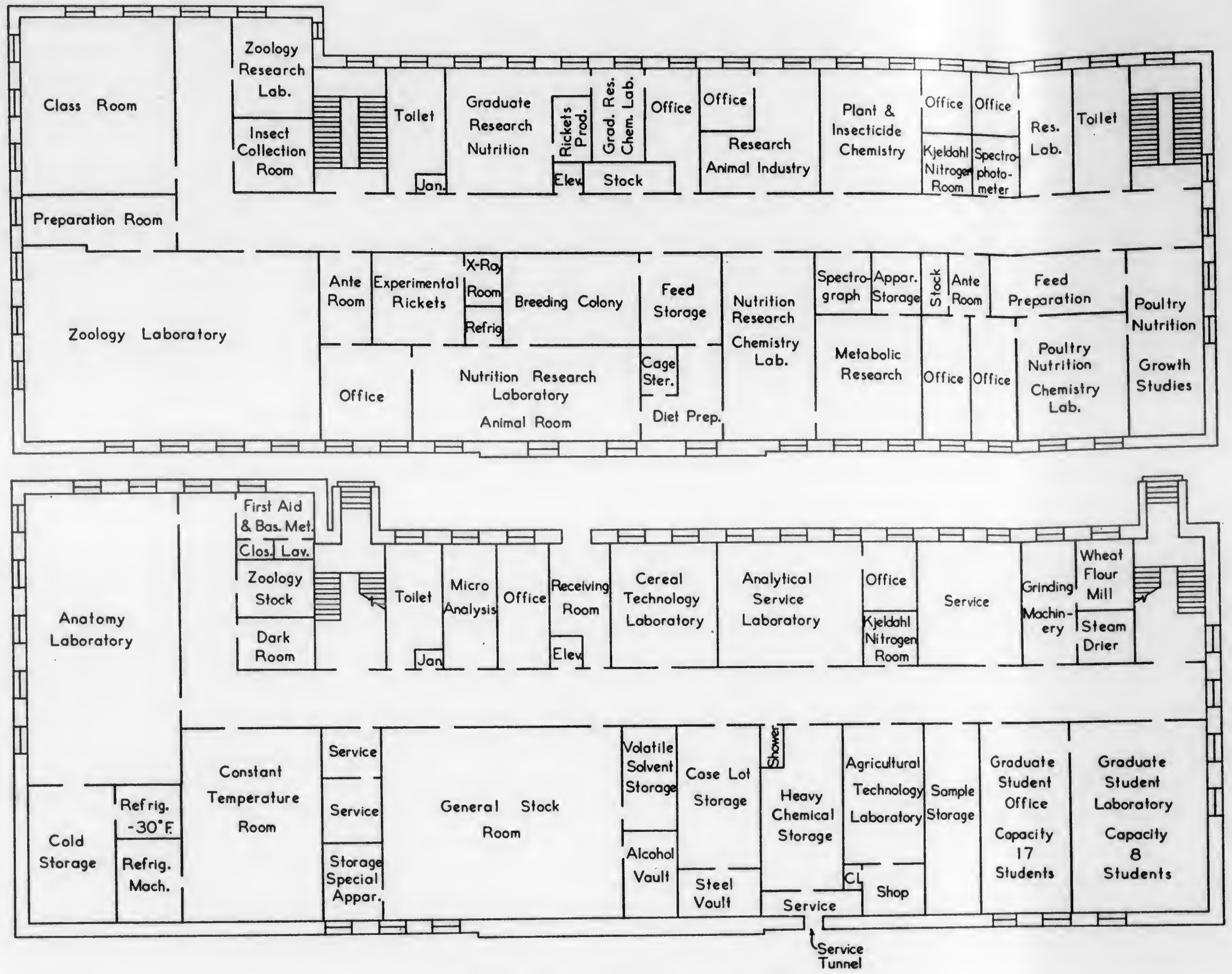
UPPER LEFT. CORNER OF PROTEIN RESEARCH LABORATORY
LOWER LEFT. GRADUATE STUDENT LABORATORY, ACCOMMODATING EIGHT STUDENTS
UPPER RIGHT. TYPICAL STUDENT LABORATORY
LOWER RIGHT. PRIVATE LABORATORY FOR TOBACCO RESEARCH

Upper Left. Laboratory for Vitamin Research
Left Center. Cereal Technology Laboratory
Lower Left. Laboratory for Research on Animal Production Problems
Upper Right. Postgraduate Work in Biophysical Chemistry
Right Center. Air-Conditioned Breeding Room, Animal Laboratory
Lower Right. Air-Conditioned Cage Room for Vitamin Research





of black linoleum tile. All laboratory desks are of wood construction with tops made of chemically treated laminated pressed wood, covered with a chemical-proof plastic finish. Desks in all laboratories are equipped with gas, cold and hot water, compressed air, vacuum lines, steam, and alternating and direct current Distilled water is conducted in aluminum tubing to laboratories and corridors from reservoirs in the attic. Forced ventilation is available for all classrooms and laboratories and all fume hoods are equipped with individual motors. Four large student laboratories, each of which will accommodate from 192 to 224 students, are designed for the various service courses in biochemistry. Balance, Kjeldahl, fat-extraction, and instrument rooms are separate from but easily accessible to the large student laboratories, as are three small classrooms, with a seating capacity of from 45 to 60. Two laboratories are set aside for graduate student research and a large office accommodates from 15 to 20 graduate students. Other graduate students have offices adjacent to private laboratories of professors with whom they are working on special problems.



Above, Third Floor; Below, Basement

Two cold rooms, maintained at 32° F., are available for quantity storage, while all laboratories are equipped with electric refrigerators for active laboratory materials. One room, for low-temperature work, is kept at −20° F. but can be used at temperatures as low as −32° F. A laboratory is also designed for pilot-plant and semicommercial-scale research in agricultural technology.

Milling facilities are available for grinding samples of every type, from rocks and soils to foods and feeds. An experimental flour mill is available for work in cereal technology. A specially designed steam dryer, made of stainless steel, has a capacity of 200 to 400 pounds for rapid drying of such materials as silage, tobacco, and agricultural crops.

A complete baking laboratory has been designed for student instruction and research. This laboratory has a baking oven with rotary hearth, fermentation cabinet (automatically controlled for temperature and humidity), dough mixers, sheeting roll, molder, and complete equipment for measuring gluten strength of flours, loaf volume, etc.

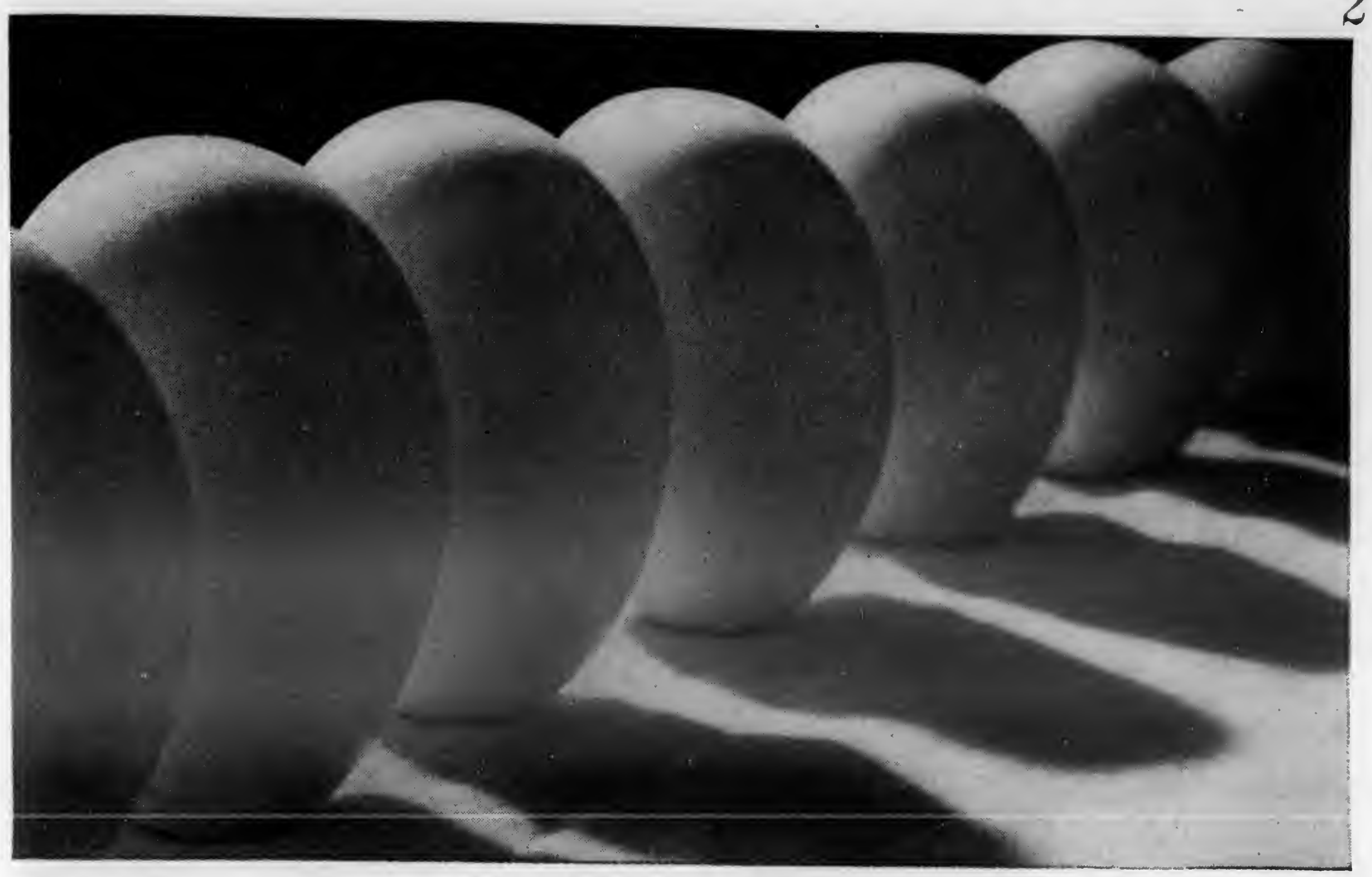
All principal storage rooms are on the basement floor. Small subsidiary stockrooms (for dispensing of stock) are located on each laboratory floor. Supplies are carried to the subsidiary stockrooms by elevator.

Experiment Station Projects

The top floor is devoted solely to research work on experiment station projects. Instrument rooms containing quartz spectrograph, spectrophotometer, fluorometers, polariscopes, and colorimeters, and an x-ray room and a darkroom for photographic work are available for all workers. Air-conditioned laboratories have been designed for animal nutrition studies and facilities are available for sterilizing all cage equipment. Battery cages are used for poultry nutrition studies and separate laboratories are available for student research in nutrition. Observation windows have been constructed so that the public can see the animals in the breeding colony and in the nutrition laboratory without entering the air-conditioned rooms and interfering with the regular research work.

The Department of Agricultural and Biological Chemistry has 108 undergraduate students majoring in the department and 33 candidates are now working for advanced degrees. The department offers service courses to approximately 1200 students from other departments and schools on the campus.

Printed in U. S. A.



Why Eggs Are Classified as a Protective Food

by R. V. BOUCHER*
Pennsylvania State College

WHAT is meant by protective foods? Why are eggs, dairy products, fruits and vegetables considered protective foods rather than sugar, lard or high patent flour? The answer is simple.

During the past 20 years great progress has been made in nutrition research, especially regarding the importance of proteins, minerals and vitamins. We know that the highly refined foods such as sugar and white flour have lost a large part of their vitamin and mineral content during the process of manufacture. Since they constitute a large part of the average diet, liberal quantities of the protective foods especially rich in the necessary vitamins, minerals and proteins must be used to balance the diet. It is the high vitamin, mineral or protein content that characterizes protective foods.

By way of an example, an analogy may be drawn between the automobile engine and man. The engine uses gasoline for fuel; man largely uses carbohydrates and fats for fuel. The spark plug is necessary to explode the gasoline thus releasing the energy for power to move the car. Vitamins play a similar role in man; vitamin B_1, for example, acts as one of the spark plugs in the human engine making the energy in starchy foods available for use by the body. Other vitamins perform equally important functions.

Minerals in protective foods play roles that are just as important as those of the vitamins. Thus, calcium and phosphorus are used to build strong bones and sound teeth. Iodine is needed to permit normal thyroid function, without which goiter results. Iron and copper are necessary for hemoglobin regenera-

---

*Dr. Boucher presented this paper at the Poultry Field Day of the New Jersey College of Agriculture June 12, 1941. For the quantitative values employed in his talk, Dr. Boucher drew freely upon current literature. As the material was presented as a talk, Dr. Boucher did not include a bibliography of the scientific literature used in its preparation.

(Reprinted from The U. S. Egg & Poultry Magazine 47 (1941) 470-473, 512.)

Distributed by the
Poultry and Egg National Board
308 West Washington Street, Chicago, Illinois

tion and if these are deficient one type of anemia is certain to develop.

Proteins are used by the body in many ways, chief among which are the growth and repair of organs and muscle tissue. To be of greatest value to the body, proteins must be of high biological value, that is, composed of the proper combination of essential amino acids.

In thinking of proteins it is helpful to visualize the protein as a brick wall and the amino acids as the bricks which are placed on top of each other to form the wall. There are 20 some different amino acids, about half of which are essential in nutrition. If a protein is composed of the proper proportion of these essential amino acids it is then said to be of high biological value.

Why are eggs classified as protective foods? A look inside the shell will furnish the answer. There we find the white weighing 58 per cent, the yolk 31 per cent, leaving the shell to account for the remaining 11 per cent.

As we all know, the yolk and the white are quite different in appearance and composition. The white contains:

Water	— 87.8	per cent
Protein	— 10.0	per cent
Ash	— 0.8	per cent
Carbohydrate	— 0.5	per cent
Fat	— 0.05	per cent

The proportion of water is high, since it serves as the principal reservoir for the embryo during incubation.

The yolk is composed of:

Water	— 49.0	per cent
Fat	— 31.6	per cent
Protein	— 16.7	per cent
Ash	— 1.5	per cent

The yolk differs widely in composition from the white, since its purpose is to supply nutritive material for the developing chick. It contains much less water and more solid materials, and is well fortified with vitamins and minerals.

Since the shell is not used for human food, its composition will be mentioned only to say that it is largely calcium carbonate — 93.7 per cent.

The percentage composition of the yolk and white combined is about:

Water	— 73.7 per cent	(37.0 grams)
Protein	— 13.4 per cent	(6.7 grams)
Fat	— 10.5 per cent	(5.2 grams)
Ash	— 1.0 per cent	(0.5 grams)
Carbohydrate	— 0.3 per cent	(0.15 grams)

The figures in parentheses represent the approximate weight of the constituents contained in an average size egg. These values emphasize the high efficiency of the modern hen as manufacturer of human food.

The 1 per cent ash contained in the egg is composed of the following chemical elements, expressed as percentage and as grams of the edible portion of an average size egg:

Calcium	0.060000 per cent	0.030000 grams
Chlorine	0.130000 per cent	0.065000 grams
Copper	0.000187 per cent	0.000094 grams
Iodine	0.000010 per cent	0.000005 grams
Iron	0.003100 per cent	0.001550 grams
Magnesium	0.050000 per cent	0.025000 grams
Manganese	0.000033 per cent	0.000017 grams
Phosphorus	0.220000 per cent	0.110000 grams
Potassium	0.140000 per cent	0.070000 grams
Sodium	0.130000 per cent	0.065000 grams
Sulfur	0.183000 per cent	.091500 grams

Smaller quanties of other elements such as zinc, silver, aluminum, tin and lead are also present. These values are not absolute for the mineral content of the egg varies to some extent depending upon the feed and the condition of the hen.

DAILY ADULT REQUIREMENTS OF ESSENTIAL NUTRIENTS

Nutrients	Daily Requirements of an Average Adult	What ONE EGG Furnishes in the Daily Diet	
Protein	70 grams	6.7 grams	or 10%
Calcium	0.8 gram	0.03 gram	or 4%
Iron	12 milligrams	1.55 milligrams	or 13%
Phosphorus	1.30 grams	110 milligrams	or 9%
Iodine	0.1 milligram	.005 milligram	or 5%
Vitamin A	5000 International Units	200 to 800 International Units	or 4 to 16%
Vitamin B_1	2 milligrams	20 to 40 International Units	or 3 to 6%
Vitamin C	75 milligrams	none	
Riboflavin	3 milligrams	100 to 200 micrograms	or 3 to 7%
Nicotinic acid	20 milligrams	about 760 micrograms	or 3 to 4%
Vitamin D	about 400 International Units	10 to 50 International Units	or 3 to 12%

Gram = 15.43 grains
Milligram = 1/1000 gram ~~or 20 International Units~~
Microgram = 1/1,000,000 gram ~~or ⅕ International Unit~~

The vitamin content of eggs also varies with the ration fed the hen. The average fresh egg, however, will contain from 200 to 800 International Units of vitamin A; 10 to 50 International Units of vitamin D; 20 to 40 International Units of Vitamin B_1, now known as thiamin; 100 to 200 micrograms of riboflavin; and 600 to 1200 micrograms of pantothenic acid, concentrated largely in the yolk. Vitamin E, vitamin K and nicotinic acid are known to be present, although in relatively smaller amounts. Vitamin B_6 is also thought to be present; however, quantitative measurements of its concentration in the egg have not been reported.

Now that we know how much of the protective food factors an egg contains, let us consider the value of an egg as protective food in a man's diet. In order to do this it is necessary to review the human requirements for the better known nutritive essentials.

Nutrients Required in Daily Diet

The Committee on Foods and Nutrition of the National Research Council and the Council on Foods of the American Medical Association have reached a tentative agreement on the daily requirements of man for the following specific nutrients:

Protein	70	grams
Calcium	0.8	gram
Iron	12	milligrams
Vitamin A	5,000	International Units
Vitamin B_1	2	milligrams
Vitamin C	75	milligrams
Riboflavin	3	milligrams
Nicotinic acid	20	milligrams
Vitamin D	Unknown	

These values are for an average size adult man who exercises to a medium degree. They do not apply to men at hard labor nor to invalids. Likewise, requirements vary for infants and children of different ages and it is known that about 400 International Units of vitamin D per day are required for pregnant and lactating women and for children.

If our present knowledge of nutrition was generally applied so that all the people in the United States were fed according to these standards, two important results would be accomplished. First, the health and vigor of the people would be greatly increased, resulting in smaller doctor and hospital bills, less loss of working time, greater efficiency and longer life. Second, from the standpoint of agriculture, there would no longer be a surplus of food products. In fact it would be necessary to increase the production of fruits, succulent vegetables and dairy and poultry products. This would mean more cows and more chickens which would in turn require more feed.

Since the approximate requirement of man for some of the more important essential nutrients is known, let us consider what part of this requirement is supplied by one egg per day. The following table gives the quantity of these nutrients contained in an average egg and the approximate precentages of the daily requirement that this quantity supplies.

Protein	6.7 grams	10%
Calcium	0.03 gram	4%
Iron	1.55 milligrams	13%
Vitamin A	200 to 800 International Units	4 to 16%
Vitamin B_1	20 to 40 International Units	3 to 6%
Vitamin C	None	——
Riboflavin	100 to 200 micrograms	3 to 7%
Nicotinic acid	About 760 micrograms	3 to 4%
Vitamin D	10 to 50 International Units	3 to 12%

Thus, we see that a single egg supplies from 3 to 16 per cent of the daily requirement of all except one of the nine essential nutrients that are most likely to be deficient in the average human diet. The one exception is vitamin C which is readily supplied by tomatoes and citrus fruits.

It must be remembered, too, that the values we have been considering are only the better known of the human requirements. The egg occupies a unique place as a food because it contains all the food elements, known and unknown, that are required to produce a perfect chick. This covers a wide range and includes all of the essential amino acids that go to form a perfect protein, as well as all the minerals and vitamins needed for embryonic growth. This is quite a severe test, for embryonic growth is both rapid and exacting in its requirements.

Ration Modifies Chemical Composition

The nutritive requirements of man and of chicks are not identical but as far as is known may be quite similar qualitatively. In many instances the quantitative requirement of the chick estimated on a body weight basis is greater than that of man.

Another point that deserves discussion is that one egg may differ from another in both physical and chemical properties to an extent which is of significance both commercially and nutritionally. From the commercial standpoint, variation in such physical characteristics as strength of shell and ratio of thick to thin white will determine the capacity of the egg to withstand transportation and storage; from the nutritional standpoint changes in certain constituents, which are measured in micrograms, will promote or inhibit the normal process of development in the embryo and influence the value of the egg in the human diet.

While the breed of the hen may affect to some extent the composition of the egg, there is ample evidence that the ration of the fowl modifies the chemical composition and, therefore, the nutritive value and hatchability of the egg. A fairly good ration will support egg production but it takes a high grade ration to produce eggs that will give high hatchability. The reason is that within certain limits the composition of the egg depends upon the feed the hen receives.

An example of this is riboflavin which, if present in the ration at a level of 600 micrograms per pound, will support high egg production but the eggs will not hatch. If the riboflavin is increased to about 1,000 micrograms per pound of feed the eggs will hatch well. It is obvious that the egg that would hatch well because of its higher riboflavin content would also be a more valuable protective food when used in a human diet.

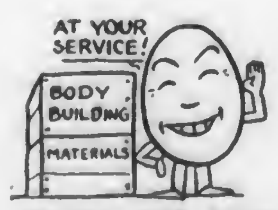
With regard to the other vitamins, it is also known that the amounts of B_1, E, K and pantothenic acid in the egg are affected by the ration.

The vitamin A potency of the egg can be increased three to four-fold. It has been shown that on practical type rations 11 to 32 per cent of the dietary intake of vitamin A is deposited in the yolk.

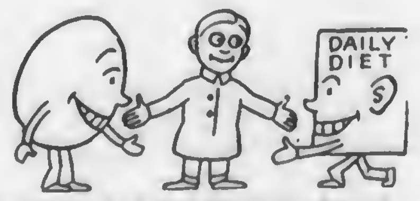
Vitamin D is transferred to the egg even more readily than vitamin A. In an experiment where massive doses of vitamin D were fed, the D content of the egg was increased 600 times resulting in a yolk that was truly a vitamin D concentrate. In an experiment using a practical type ration containing 1 per cent cod liver oil, it was found that 10 per cent of the vitamin D in the feed was transferred to the egg. On this basis, the hen evidently transmits dietary vitamin D to the egg more efficiently than the cow transfers it to milk since it was found that only 2 per cent of the D in cows feed appeared in the milk.

Thus we can say that the composition of the hen's egg is not constant. This is important since it affects the nutritional value, hatchability and ability of the egg to stand up under transportation and storage.

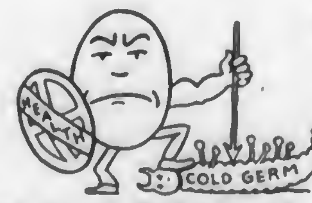
What would be the effect if everybody in the nation ate one egg per day? That would be a total of 47,450 million eggs per year, which is 10,818 million more eggs per year than were produced on the average during the 1925-1937 period. To produce that number of eggs would require an increase of about 30 per cent in nation-wide egg production.



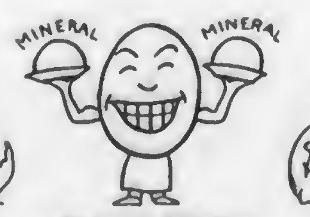
PROTEIN FOR BODY BUILDING



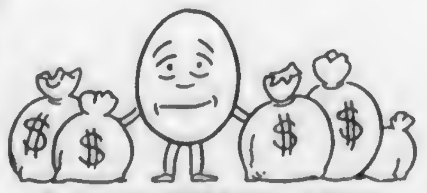
DOCTORS RECOMMEND EGGS



BUILD UP RESISTANCE



BUILD SOUND TEETH



RICH IN ORGANIC IRON

RICH IN VITAMIN D

MANGANESE IN ANIMAL NUTRITION

With Special Reference to PIGS

By

R. C. Miller and T. B. Keith

Pennsylvania State College

* * *

★ ★ Reprinted from FEEDSTUFFS of August 30, 1941 ★ ★

MANGANESE *in* Animal Nutrition

With Special Reference to PIGS

★ ★

By R. C. Miller and T. B. Keith
Pennsylvania State College

FOR many years there has been a growing interest in the role played by the element manganese in the animal body. Among the earlier findings of importance is that of Bertrand and Medigreceanu, who in 1913 found that manganese is normally present in the blood and organs of man and the higher animals. The mere presence of an element in a tissue or organ does not necessarily mean that it has an essential function but it does indicate that such a role is possible. In the case of manganese an understanding of its function is not complete even today, although research has revealed several relationships in which it is important.

Many workers have simply considered manganese as a trace element, small amounts of which are necessary for one or more body functions. Definite proof of the vital function of manganese has been difficult, however, chiefly because of the difficulty of obtaining diets for experimental feeding which are free of this element.

As a result of research several essential functions of manganese have been suggested in the past few years and it is now regarded as being necessary for complete body development and the continuance of certain body functions. While only small amounts of manganese are required in the diet, such amounts appear to be absolutely essential although the exact mode of function of the element is not fully understood.

There is considerable evidence to show that manganese is necessary for the normal reproduction of animals. In cases of a lack of manganese there have been reported such disorders as disturbances of the estrus cycle, delayed sexual maturity, irregular ovulation, failure of mammary development and testicular degeneration. While there is some divergence of opinion perhaps as to all of the exact conditions resulting from a lack of manganese, the fact seems to be well established that this element is necessary for the fulfillment of the complete reproductive cycle.

In the past few years much attention has been focused on the role played by manganese in poultry nutrition, particularly in relation to perosis. This is a disorder in which there is an enlargement and flattening of the hock joint accompanied by the slipping of the tendon from its normal position. It has been reported that the addition of manganese to the diet to bring the manganese content up to 40-60 parts per million, is efficacious in most cases in preventing perosis. Recently, however, it has been indicated that manganese may not be the only factor involved in the production and prevention of perosis, rather it is one of several factors involved in this syndrome.

GROWTH OF CHICKS

In addition to the role played in perosis, manganese has been reported as being necessary for the growth of chicks, the development of chick bones, the maintenance of the body weight of hens, egg production, egg shell strength and the hatchability of eggs.

More recently it has been indicated that manganese may play a role in the nutrition of swine. In this connection stiffness or lameness was found to occur when pigs were fed rations low in manganese content. This disorder was found to occur on a number of rations all of which had as components corn, tankage, soybean oil meal, alfalfa and salt.

Approximately half of the pigs fed these rations developed the outward symptoms at about the time when they weighed 150 lbs. In addition to the stiffness, the hock joints were enlarged and many of the pigs had crooked legs. In outward appearance the condition resembled rickets, but x-rays showed that there was no mineral depletion of the leg bones, neither was there any lack of calcium and phosphorus or vitamin D in the diet.

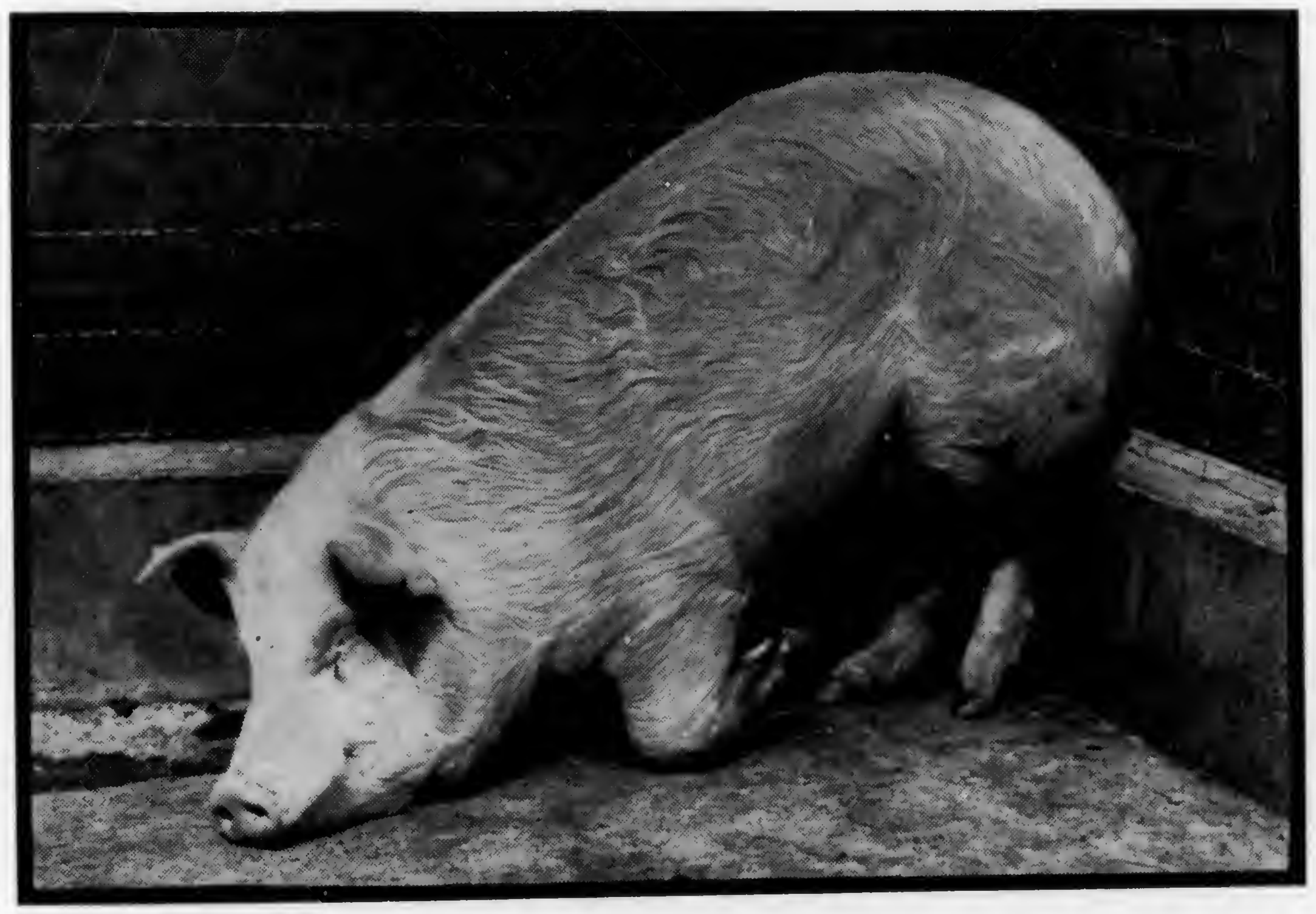
On the other hand, the total mineral content of the rations on which stiffness occurred was found to be relatively high, ranging from 8 to 12%, while the manganese content was relatively low, 11 parts per million. Bringing the manganese content of these rations up to 50-60 parts per million by the addition of manganese sulfate resulted in pigs which developed normally with no stiffness. However, the stiffness could not be cured by the addition of manganese after it had once occurred.

The growth of the pigs fed the low manganese rations did not seem to be impaired and manganese itself is not usually considered as influencing growth, at least in the first generation. Experiments conducted in this country and in England support this point of view. The dispensability of manganese in succeeding generations is a subject requiring further study and one which will be difficult because of the necessity of separating the manganese requirement for growth from that for reproduction.

The question may be raised as to whether or not the requirement for manganese is influenced by or dependent upon conditions invoked by other dietary factors. The lameness of pigs which has been described was produced while feeding rations relatively high in mineral content. It is possible with the emphasis that has been placed in recent years on the feeding of minerals to swine, that many pigs may be receiving excessive amounts of calcium and phosphorus which may result in the hypercalcification of the bones as manifested by enlarged joints, crooked legs and lameness all of which are commonly seen. Recent complaints from packers indicate that many pigs now coming to market have very hard bones.

It is not at all unusual to find pigs being fed rations which are high in mineral content. An inspection of analyses of commercial hog feeds, as published by various feed control laboratories, shows that many commercial hog feeds contain as much as 8 to 10% of minerals. It may be possible that additional manganese is required when feeding rations supplying relatively high amounts of calcium and phosphorus and relatively low amounts of manganese.

The question may be asked as to how much manganese is normally found in feedstuffs. Quite commonly it is stated that manganese is widely distributed in water, plants and animals. While some manganese is found in practically every plant, the amount present may be fairly



Stiffness or lameness has been found to occur when pigs are fed rations low in manganese content. In addition to the stiffness, the hock joints were enlarged and many of the pigs had crooked legs.

constant for a particular plant or it may vary widely. Oats have been reported to contain from 5 to 290 parts per million of manganese.

Corn which predominates in most hog rations contains less manganese than almost any other feedstuff, usually about 5 parts per million. Soybeans usually contain 25-35 parts per million, alfalfa 30-36 parts and tankage 20-30 parts. The manganese content of milk is almost negligible. Wheat itself contains about 30 parts per million of manganese, while wheat by-products such as bran and middlings may contain approximately 100 parts per million. Forages as a rule contain two to three times as much manganese as do most concentrates, but the manganese content of any plant depends on the conditions under which it is grown. In the first place manganese must be present in the soil either naturally or through fertilization.

There are localities in which the soil is unproductive unless it is fertilized with manganese. However, the presence of manganese in the soil does not insure its assimilation by the plant. The soil must be acid for manganese to be available to the plant and, furthermore, the best assimilation of manganese occurs when conditions in the soil favor reduction. Most feed crops and forages are not produced where conditions such as these exist and the result may be feeds of relatively low manganese content.

The importance of soil, plant and animal inter-relationships is well illustrated in the case of manganese and may assume added significance as the true physiological functions of this element are revealed through further research.

THE ESSENTIAL NATURE OF MANGANESE TO ALL LIFE

MANGANESE is the element responsible for growth and reproduction. Its essential nature to life is conceded and can be verified by practical experiments.

It is required in soil reaction, as a plant hormone, and in all animal life, either as a chemical or catalyst.

There is no form of life that can exist without manganese. We deal, therefore, with a subject of which we shall never know too much.

The article in the foregoing pages is reprinted and available to the trade with our compliments and our own publication, "Manganese—Its Value in Soil, Plant and Animal Nutrition, July, 1939," will be furnished free to those interested while the edition lasts.

THE HARSHAW CHEMICAL COMPANY
Cleveland, Ohio

September 8, 1941

MANGANESE SULPHATE

PURIFIED — FEED GRADE

Harshaw manganese sulphate purified—feed grade is especially prepared for addition to feeds. It is a high grade compound, readily soluble in water, therefore, easily assimilated by the animal body, and is excellent in metabolic efficiency for correcting a manganese deficiency in animal nutrition. . . . Manganese is one of the essential elements to all life and is particularly required for growth and reproduction, bone formation, and general health. Feeds that are lacking or deficient in this necessary element should be corrected by the addition of Harshaw's Manganese Sulphate—Feed grade. . . .

THE HARSHAW CHEMICAL CO.

Cleveland, Ohio, and Principal Cities

Ask for Price List and the following Booklets and Articles:

The Status of Minor Elements in Liebig's Day and Recent Developments.
By L. G. Willis

Many-Sided Manganese.
By L. C. Norris

Mineral Hunger in Live Stock.
By R. H. Lush

Cobalt in the Treatment and Prevention of Salt Sick.
By W. M. Neal

The Role of Minerals in Bang's Disease of Cattle.
By Irene Levis and F. H. Emery

Balanced Minerals Essential in Good Feed Ration.
By B. W. Fairbanks

General Pacific Coast Representatives, Wilson & George Meyer & Company, San Francisco, Calif.

A Comparison of Ph
Under Practical Co

Young ringnecks in brooder house at Loyalsock State Game

IN recent years research workers at The Pennsylvania State College have developed a number of satisfactory starting rations for ringneck pheasants *(Phasianus colchicus torquatus)*. The rations have been tested thoroughly under carefully controlled experimental conditions. Until the work reported
here was u
been possi
mercial

In th
tion
ma
T

ed by the regular b
The experimental
weeks.

Each group was
brooder house, 12
electric brooder, w
ing equipment wer

s and exercising themselves in the yard adjacent to the brooder house.

quart of molasses with ten gal
This mildly laxative drink w
precaution against coccidiosis
symptoms of coccidiosis appear
course of the experiment.

Data

The chicks were weighed by
day and individually when
weeks of age. At the time of e
weighing every chick was
symptoms of perosis. For purp
fication the chicks were indiv
banded when two weeks of a
sumption records were kept
basis. Since all groups fed eg
received the same quantity b
record of the consumption of
ments was kept. Consequent
sumption as used in this paper
intake. The amount of supple
was such that it was complete
relatively short time. Mortalit
ed daily.

Results and Discuss

The records of this expe
studied statistically by analysi
(Snedecor, 1937). Variations b
groups fed the same ration we
insignificant. For this reason
be made only to rations in
results.

Average body weights of a
shown in table 3. These wei
adjusted for sex variation si
farm program necessitated tr
birds at six weeks of age from b
ters to covered fields containin
ants. Catching the pheasants f
inspection was impractical un
ditions. Accurate sexing by e
ination is not possible for bi
However, statistical analysis
much smaller groups of phea
previous experiments, demo
equally as accurate compariso
pected from using either unad
justed weight figures for six-w
Table 4 ranks all groups accord
body weight. Comparisons were
rations 1, 2, and 3 and among
and 3s. Rations 1, 2,, and 3 w
pared with rations 1s, 2s, and 3s
All weight variations were sh
significant.

Weekly feed consumption p
culated on a 100 bird basis, is s
5. The feeding of eggs and le
decrease in feed consumption
case of ration 3s. The amou
consumed was significantly gre
amount of ration 3s. Variation
sumption between rations 1 a
tween 2 and 2s were insignific
sumption of ration 1 was signifi

MANGANESE SULPHATE
PURIFIED — FEED GRADE

Harshaw manganese sulphate purified—feed grade is especially prepared for addition to feeds. It is a high grade compound, readily soluble in water, therefore, easily assimilated by the animal body, and is excellent in metabolic efficiency for correcting a manganese deficiency in animal nutrition. . . . Manganese is one of the essential elements to all life and is particularly required for growth and reproduction, bone formation, and general health. Feeds that are lacking or deficient in this necessary element should be corrected by the addition of Harshaw's Manganese Sulphate—Feed grade. . . .

Ask for Price List and the following Booklets and Articles:

The Status of Minor Elements in Liebig's Day and Recent Developments.
By L. G. Willis

Many-Sided Manganese.
By L. C. Norris

Mineral Hunger in Live Stock.
By R. H. Lush

Cobalt in the Treatment and Prevention of Salt Sick.
By W. M. Neal

The Role of Minerals in Bang's Disease of Cattle.
By Irene Levis and F. H. Emery

Balanced Minerals Essential in Good Feed Ration.
By B. W. Fairbanks

THE HARSHAW CHEMICAL CO.
Cleveland, Ohio, and Principal Cities

General Pacific Coast Representatives, Wilson & George Meyer & Company, San Francisco, Calif.

A Comparison of Pheasant Starting Rations Under Practical Commercial Conditions[*]

Young ringnecks in brooder house at Loyalsock State Game Farm.

By W. J. STADELMAN[‡]
R. R. MURPHY
E. W. CALLENBACH
R. V. BOUCHER[†]

Reprinted with permission of the Pennsylvania Agricultural Experimental Station from the August, 1941 issue of the Pennsylvania Game News, Vol. XII, No. 5, pp. 4-5 and 28-29.

★

IN recent years research workers at The Pennsylvania State College have developed a number of satisfactory starting rations for ringneck pheasants (*Phasianus colchicus torquatus*). The rations have been tested thoroughly under carefully controlled experimental conditions. Until the work reported here was undertaken, however, it had not been possible to study the rations under commercial conditions.

In the summer of 1940 the active cooperation of the Pennsylvania Game Commission made possible two large scale experiments. These have provided, under commercial conditions of propagation, comparative information on three acceptable pheasant starting mash mixtures. The experiments were conducted at the Loyalsock State Game Farm, Montoursville, Pennsylvania.

EXPERIMENT ONE

The experiment was started on May 23, 1940. It consisted of two series of six groups each, a total of twelve groups. Each group contained 280 day-old ringneck pheasant chicks. No attempt was made to secure random samples of chicks. All chicks were hatched at the game farm from eggs produced by the regular breeding stock kept there. The experimental brooding period was six weeks.

Each group was brooded in a circular brooder house, 12 feet in diameter. The electric brooder, water fountains, and feeding equipment were the same as those used in all 80 pheasant brooder houses on the farm. The usual farm routine of feeding, watering and housecleaning was followed. After the chicks were one week old, weather permitting, they were allowed to range in a yard, about 100 square feet in area. At two weeks of age they were given access to a yard having an area of approximately 6,000 square feet. A legume sod mixed with cereal grains was maintained in all yards.

Rations and Feeding System

In 1934, Callenbach, Hunter and Murphy formulated a successful 28 per cent protein pheasant starting mash. In order to provide this high level of protein, a special 65 per cent protein meat scraps was used instead of the more usual meat scraps of 50 to 55 per cent protein content. Unfortunately, some millers using the mash formula substituted for the 65 per cent protein meat scraps the more easily obtainable meat scraps of lower protein and higher mineral content. The result has been serious difficulties from perosis. To obviate this situation, Skoglund, in 1940, developed a satisfactory starting mash, utilizing soybean oil meal and 50 per cent protein meat scraps as sources of supplementary protein.

In this experiment, the mash mixture developed by Skoglund (1940) was designated mash mixture 1 and was fed to groups 1, 2, 7 and 8. Groups 7 and 8 also received daily feedings of chopped hard-boiled pheasant eggs for the first three weeks and chopped lettuce throughout the experiment. Groups 3, 4, 9 and 10 were fed the original mash mixture formulated by Callenbach and co-workers (1934), and designated mash mixture 2. This mixture has been used for six years at the Loyalsock State Game Farm with excellent results. The supplements fed groups 7 and 8 were also given groups 9 and 10. Mash mixture 3 in this experiment was a commercial closed formula pheasant starting mash which is prepared in granular form. This mixture had been tested in previous years and was found to be satisfactory. It was fed to groups 5, 6, 11 and 12 with groups 11 and 12 receiving the same supplements as groups 7, 8, 9 and 10. The formulae of mash mixtures 1 and 2 are shown in table 1. The formula of mash mixture 3 is not known. Table 2 gives the chemical analyses of the three mash mixtures.

The schedule for feeding the mash mixtures and supplements is indicated in the following outline. Ration number indicates the mash mixture fed. The letter "s" indicates a particular mash mixture was supplemented with eggs and lettuce.

Group Number	Ration Number	Ration Fed Supplement
1	1	None
2	1	None
3	2	None
4	2	None
5	3	None
6	3	None
7	1s	Eggs and lettuce*
8	1s	Eggs and lettuce*
9	2s	Eggs and lettuce*
10	2s	Eggs and lettuce*
11	3s	Eggs and lettuce*
12	3s	Eggs and lettuce*

*Chopped hard-boiled eggs for first three weeks and chopped lettuce for entire experiment; daily feedings.

All groups had constant access to fresh water and granite grit except that, beginning with the second week and each week thereafter, the water was replaced for one entire day per week with a dilute molasses solution. The solution was prepared by mixing one quart of molasses with ten gallons of water. This mildly laxative drink was given as a precaution against coccidiosis infestation. No symptoms of coccidiosis appeared during the course of the experiment.

---

[*] Paper 27 from the Pennsylvania Cooperative Wildlife Research Unit; Fish and Wildlife Service (U. S. Department of the Interior), The Pennsylvania State College, Pennsylvania Game Commission, and the American Wildlife Institute Cooperating.

[**] Authorized for publication on June 4, 1941 as paper No. 1034 in the journal series of the Pennsylvania Agricultural Experiment Station.

[‡] Graduate Assistant in Poultry Husbandry from the Pennsylvania Cooperative Wildlife Research Unit.

[†] Assistant Professor of Poultry Husbandry, Professor of Poultry Husbandry, and Associate Professor of Agricultural and Biological Chemistry, respectively.

Data

The chicks were weighed by groups at one day and individually when four and six weeks of age. At the time of each individual weighing every chick was examined for symptoms of perosis. For purposes of identification the chicks were individually wing-banded when two weeks of age. Mash consumption records were kept on a weekly basis. Since all groups fed eggs and lettuce received the same quantity by weight, no record of the consumption of these supplements was kept. Consequently, feed consumption as used in this paper refers to mash intake. The amount of supplementary feed was such that it was completely eaten in a relatively short time. Mortality was recorded daily.

Results and Discussion

The records of this experiment were studied statistically by analysis of variance (Snedecor, 1937). Variations between paired groups fed the same ration were found to be insignificant. For this reason reference will be made only to rations in reporting the results.

Average body weights of all groups are shown in table 3. These weights were not adjusted for sex variation since the game farm program necessitated transfer of the birds at six weeks of age from brooding quarters to covered fields containing other pheasants. Catching the pheasants for weighing or inspection was impractical under these conditions. Accurate sexing by external examination is not possible for birds so young. However, statistical analysis of data from much smaller groups of pheasants used in previous experiments, demonstrated that equally as accurate comparisons may be expected from using either unadjusted or adjusted weight figures for six-week old birds. Table 4 ranks all groups according to average body weight. Comparisons were made among rations 1, 2, and 3 and among rations 1s, 2s, and 3s. Rations 1, 2,, and 3 were also compared with rations 1s, 2s, and 3s, respectively. All weight variations were shown to be insignificant.

Weekly feed consumption per group, calculated on a 100 bird basis, is shown in table 5. The feeding of eggs and lettuce caused a decrease in feed consumption only in the case of ration 3s. The amount of ration 3 consumed was significantly greater than the amount of ration 3s. Variations in tòtal consumption between rations 1 and 1s and between 2 and 2s were insignificant. The consumption of ration 1 was significantly greater than that of ration 2. The variation between rations 1s and 2s was insignificant.

The amounts of rations 3 and 3s consumed were very significantly less than those of rations 1 or 2 and 1s and 2s, respectively. Wastage was probably largely responsible for these significant differences. The chicks fed the mashes in rations 1, 2, 1s, and 2s, threw considerably more feed out of the hoppers than chicks fed the granular mixture in rations 3 and 3s. No measurement of wastage was made, but observations of feed in the litter around hoppers lend weight to the suggestion.

Table 6 shows group relationships between feed consumption and gain in body weight. As indicated below in the discussion of mortality, some birds which escaped from the several groups were returned to other groups. Thus, feed consumption and feed efficiency data are not completely accurate. They are, however, definitely comparable. Ration 2 was slightly more efficient than ration 1. Variations between rations 1 and 1s, 2 and 2s, 3 and 3s and 1s and 2s were insignificant. Variations between rations 1 and 3, 2 and 3, 1s and 3s and 2s and 3s were highly significant in favor of rations 3 and 3s in all comparisons. Wastage of mash as in feed consumption per 100 chicks, also had considerable influence on the amount of feed required to produce a pound of gain in body weight in those groups fed rations 1, 1s, 2 and 2s.

Variations in mortality between groups, as shown in table 7, were insignificant. The figures in table 7 are listed as per cent mortality on a weekly basis. For analysis of variation the original data were used, only the recorded mortality being considered. Birds listed as lost or gained were not charged against the respective groups. These transfers between groups were caused by birds flying over the outside fences and not being returned to the proper pens.

The cost of pheasant chicks reared is dependent, in large part, on the cost of feed. Cost per bird and cost per pound of body gain for mash feed consumed were calculated and are given in table 8. These figures are based on feed prices as of June 1, 1940, when mash mixture 1 was purchasable for $55.10, mixture 2 for $60.39, and mixture 3 for $86.40 per ton. No significant differences were found in cost of mash per bird between the groups fed the same mash mixtures, either with or without hard-boiled eggs and lettuce as a supplement. In the analysis of figures given in table 8, rations 1 and 1s were significantly more economical than rations 3 and 3s. Slightly significant differences in cost per bird were found when rations 2 and 2s were compared with rations 3 and 3s. The differences favored rations 2 and 2s. Rations 1, 1s, 2, and 2s were more economical than rations 3 and 3s in producing a pound of body gain. No significant variation was found between rations 1, 1s, 2 and 2s when compared in all possible combinations. From these data it appears that even with the wastage of mash mixtures 1 and 2, they were more economical than mash mixture 3. The results obtained from the feeding of hard-boiled eggs and lettuce, in addition to the mash mixtures, indicate that the practice did not justify the expense involved.

EXPERIMENT TWO

On July 11, 1940, 240 day-old ringneck pheasant chicks were placed in each of the same twelve broder houses used in Experiment One. This time the chicks were carefully selected by random sample, some from each incubator tray being placed in each group. The identification, management and feeding of the chicks were the same as in Experiment One. Although new yards were used, the sod was less abundant than for the earlier chicks. Since no cases of perosis were observed in the first experiment, the chicks in Experiment Two were not individually examined for symptoms of this nutritional disorder. In all respects other than those just indicated, Experiment Two was a duplication of Experiment One.

Results and Discussion

The data were analyzed as described previously. As in Experiment One, no significant differences were found to exist between paired groups, receiving identical rations, in any of the ways compared.

Average body weights and ranking of all groups are shown, with comparable figures for Experiment One, in tables 3 and 4. The weights and placements were determined on the same basis as in the previous experiment. Variations between rations, compared in all possible combinations, were, as in Experiment One, insignificant.

Weekly feed consumption is again presented in table 5. Supplementary feeding of hard-boiled eggs and lettuce resulted in a significant reduction in mash consumption only with mash mixture 1. A significantly greater amount of ration 1 was consumed than of ration 2. The amounts of rations 3 and 3s consumed were found to be considerably less than of rations 1, 2, 1s and 2s. As in Experiment One, the groups fed rations 3 and 3s wasted considerably less feed than did groups fed the other rations. No measurement of feed wastage was made.

Young pheasants sunning and exercising themselves in the yard adjacent to the brooder house.

The relation of feed consumed to gain in weight is shown in table 6. Variation between rations 1s and 2s, 2 and 2s, and 3 and 3s were insignificant. Rations 1s, 2 and 3 were found to be more efficiently utilized than ration 1. Ration 3s was significantly more efficient than rations 1s or 2s. Variation between rations 2 and 3 was found to be significant in favor of ration 3.

Analysis of the mortality data, table 7, by the same method as used in Experiment One showed all variations to be insignificant. For late hatched chicks, the mortality was very satisfactory. More trouble was experienced from escaped birds, there being one pen with more birds at the end than at the beginning of the experiment. It is not known why so many birds were lost and unaccounted for in group 2.

Feed costs calculated either as mash consumed per bird or per pound of gain were similar to results of Experiment One, as indicated in table 8. In both experiments mash mixtures 1 and 2 were significantly more economical than mash mixture 3. Variation between groups fed mash mixtures 1 and 2 were statistically insignificant in both experiments, but the trend in both cases was in favor of mash mixture 1.

Summary

1. Two experiments of 12 groups each, involving a total of 6,240 ringneck pheasant chicks, were conducted under practical, commercial conditions at the Loyalsock Game Farm. Three mash mixtures, which varied considerably in cost, were compared. The mash mixtures were fed as complete rations and with supplements of hard-boiled eggs and lettuce.
2. Comparisons were made of body weights, feed efficiency, feed consumption, feed costs and mortality.
3. All three mash mixtures were found to be adequate and satisfactory for producing desirable six-week
Mortality in all groups w
ering was excellent.

Sayre Sportsn

4. Rations 1, 1s, 2 a
ical than rations
individual bird
of gain in body
5. Supplementary
eggs and lettuce
mixture apparen
quality or quan
increases labor

Ackno

To Richard Gers
Commission, for his
planning and condu

To Earl S. Green
the Loyalsock State
operation in condu
keeping of records.

To the workers
Game Farm for the
of data and for e

quart of molasses with ten gallons of water. This mildly laxative drink was given as a precaution against coccidiosis infestation. No symptoms of coccidiosis appeared during the course of the experiment.

Data

The chicks were weighed by groups at one day and individually when four and six weeks of age. At the time of each individual weighing every chick was examined for symptoms of perosis. For purposes of identification the chicks were individually wing-banded when two weeks of age. Mash consumption records were kept on a weekly basis. Since all groups fed eggs and lettuce received the same quantity by weight, no record of the consumption of these supplements was kept. Consequently, feed consumption as used in this paper refers to mash intake. The amount of supplementary feed was such that it was completely eaten in a relatively short time. Mortality was recorded daily.

Results and Discussion

The records of this experiment were studied statistically by analysis of variance (Snedecor, 1937). Variations between paired groups fed the same ration were found to be insignificant. For this reason reference will be made only to rations in reporting the results.

Average body weights of all groups are shown in table 3. These weights were not adjusted for sex variation since the game farm program necessitated transfer of the birds at six weeks of age from brooding quarters to covered fields containing other pheasants. Catching the pheasants for weighing or inspection was impractical under these conditions. Accurate sexing by external examination is not possible for birds so young. However, statistical analysis of data from much smaller groups of pheasants used in previous experiments, demonstrated that equally as accurate comparisons may be expected from using either unadjusted or adjusted weight figures for six-week old birds. Table 4 ranks all groups according to average body weight. Comparisons were made among rations 1, 2, and 3 and among rations 1s, 2s, and 3s. Rations 1, 2,, and 3 were also compared with rations 1s, 2s, and 3s, respectively. All weight variations were shown to be insignificant.

Weekly feed consumption per group, calculated on a 100 bird basis, is shown in table 5. The feeding of eggs and lettuce caused a decrease in feed consumption only in the case of ration 3s. The amount of ration 3 consumed was significantly greater than the amount of ration 3s. Variations in total consumption between rations 1 and 1s and between 2 and 2s were insignificant. The consumption of ration 1 was significantly greater than that of ration 2. The variation between rations 1s and 2s was insignificant.

The amounts of rations 3 and 3s consumed were very significantly less than those of rations 1 or 2 and 1s and 2s, respectively. Wastage was probably largely responsible for these significant differences. The chicks fed the mashes in rations 1, 2, 1s, and 2s, threw considerably more feed out of the hoppers than chicks fed the granular mixture in rations 3 and 3s. No measurement of wastage was made, but observations of feed in the litter around hoppers lend weight to the suggestion.

Table 6 shows group relationships between feed consumption and gain in body weight. As indicated below in the discussion of mortality, some birds which escaped from the several groups were returned to other groups. Thus, feed consumption and feed efficiency data are not completely accurate. They are, however, definitely comparable. Ration 2 was slightly more efficient than ration 1. Variations between rations 1 and 1s, 2 and 2s, 3 and 3s and 1s and 2s were insignificant. Variations between rations 1 and 3, 2 and 3, 1s and 3s and 2s and 3s were highly significant in favor of rations 3 and 3s in all comparisons. Wastage of mash as in feed consumption per 100 chicks, also had considerable influence on the amount of feed required to produce a pound of gain in body weight in those groups fed rations 1, 1s, 2 and 2s.

Variations in mortality between groups, as shown in table 7, were insignificant. The figures in table 7 are listed as per cent mortality on a weekly basis. For analysis of variation the original data were used, only the recorded mortality being considered. Birds listed as lost or gained were not charged against the respective groups. These transfers between groups were caused by birds flying over the outside fences and not being returned to the proper pens.

The cost of pheasant chicks reared is dependent, in large part, on the cost of feed. Cost per bird and cost per pound of body gain for mash feed consumed were calculated and are given in table 8. These figures are based on feed prices as of June 1, 1940, when mash mixture 1 was purchasable for $55.10, mixture 2 for $60.39, and mixture 3 for $86.40 per ton. No significant differences were found in cost of mash per bird between the groups fed the same mash mixtures, either with or without hard-boiled eggs and lettuce as a supplement. In the analysis of figures given in table 8, rations 1 and 1s were significantly more economical than rations 3 and 3s. Slightly significant differences in cost per bird were found when rations 2 and 2s were compared with rations 3 and 3s. The differences favored rations 2 and 2s. Rations 1, 1s, 2, and 2s were more economical than rations 3 and 3s in producing a pound of body gain. No significant variation was found between rations 1, 1s, 2 and 2s when compared in all possible combinations. From these data it appears that even with the wastage of mash mixtures 1 and 2, they were more economical than mash mixture 3. The results obtained from the feeding of hard-boiled eggs and lettuce, in addition to the mash mixtures, indicate that the practice did not justify the expense involved.

EXPERIMENT TWO

On July 11, 1940, 240 day-old ringneck pheasant chicks were placed in each of the same twelve broder houses used in Experiment One. This time the chicks were carefully selected by random sample, some from each incubator tray being placed in each group. The identification, management and feeding of the chicks were the same as in Experiment One. Although new yards were used, the sod was less abundant than for the earlier chicks. Since no cases of perosis were observed in the first experiment, the chicks in Experiment Two were not individually examined for symptoms of this nutritional disorder. In all respects other than those just indicated, Experiment Two was a duplication of Experiment One.

Results and Discussion

The data were analyzed as described previously. As in Experiment One, no significant differences were found to exist between paired groups, receiving identical rations, in any of the ways compared.

Average body weights and ranking of all groups are shown, with comparable figures for Experiment One, in tables 3 and 4. The weights and placements were determined on the same basis as in the previous experiment. Variations between rations, compared in all possible combinations, were, as in Experiment One, insignificant.

Weekly feed consumption is again presented in table 5. Supplementary feeding of hard-boiled eggs and lettuce resulted in a significant reduction in mash consumption only with mash mixture 1. A significantly greater amount of ration 1 was consumed than of ration 2. The amounts of rations 3 and 3s consumed were found to be considerably less than of rations 1, 2, 1s and 2s. As in Experiment One, the groups fed rations 3 and 3s wasted considerably less feed than did groups fed the other rations. No measurement of feed wastage was made.

Young pheasants sunning and exercising themselves in the yard adjacent to the brooder house.

The relation of feed consumed to gain in weight is shown in table 6. Variation between rations 1s and 2s, 2 and 2s, and 3 and 3s were insignificant. Rations 1s, 2 and 3 were found to be more efficiently utilized than ration 1. Ration 3s was significantly more efficient than rations 1s or 2s. Variation between rations 2 and 3 was found to be significant in favor of ration 3.

Analysis of the mortality data, table 7, by the same method as used in Experiment One showed all variations to be insignificant. For late hatched chicks, the mortality was very satisfactory. More trouble was experienced from escaped birds, there being one pen with more birds at the end than at the beginning of the experiment. It is not known why so many birds were lost and unaccounted for in group 2.

Feed costs calculated either as mash consumed per bird or per pound of gain were similar to results of Experiment One, as indicated in table 8. In both experiments mash mixtures 1 and 2 were significantly more economical than mash mixture 3. Variation between groups fed mash mixtures 1 and 2 were statistically insignificant in both experiments, but the trend in both cases was in favor of mash mixture 1.

Summary

1. Two experiments of 12 groups each, involving a total of 6,240 ringneck pheasant chicks, were conducted under practical, commercial conditions at the Loyalsock Game Farm. Three mash mixtures, which varied considerably in cost, were compared. The mash mixtures were fed as complete rations and with supplements of hard-boiled eggs and lettuce.
2. Comparisons were made of body weights, feed efficiency, feed consumption, feed costs and mortality.
3. All three mash mixtures were found to be adequate and satisfactory for producing desirable six-week old pheasants. Mortality in all groups was low and feathering was excellent.
4. Rations 1, 1s, 2 and 2s were more economical than rations 3 to 3s in producing an individual bird or in producing a pound of gain in body weight.
5. Supplementary feeding of hard-boiled eggs and lettuce with an adequate mash mixture apparently has no effect on the quality or quantity of birds raised and increases labor and feed costs.

Photo by Donald Hicks

Sayre Sportsmen's Association, pheasant pens. Birds being wintered for Spring release.

Acknowledgments

To Richard Gerstell, Pennsylvania Game Commission, for his complete cooperation in planning and conducting the experiment.

To Earl S. Greenwood, superintendent of the Loyalsock State Game Farm, for his cooperation in conducting the work and in keeping of records.

To the workers of the Loyalsock State Game Farm for their assistance in collection of data and for excellent care given the chicks throughout the two experiments.

Literature Cited

Callenbach, E. W., J. E. Hunter, R. R. Murphy
1940. Unpublished data.
Skoglund, W. C.
1940. An Improved Ration for Starting Ringneck Pheasants. Pennsylvania Experiment Station Bulletin No. 389.
Snedecor, G. W.
1937. Statistical Methods. Collegiate Press, Inc., Ames, Iowa.

Table 1.—Formulae of mash mixtures.

Ingredients	Mixture 1 pounds	Mixture 2 pounds
Ground yellow corn	11.2	20.0
Wheat bran	15.0	15.0
Flour wheat middlings	12.5	12.5
Ground heavy oats	10.0	10.0
Dry skim milk	12.5	12.5
Alfalfa leaf meal	5.0	5.0
White fish meal	2.75	11.0
Salt	.5	.5
Cod liver oil (400 A.O.A.C. chick units Vitamin D per gram)	.25	.25
Meat scraps (65% protein)	....	13.5
Meat scraps (50% protein)	11.05	....
Soybean oil mash	19.5	....
Manganese sulfate	.025	.025
Total	100.275	100.275

Table 2.—Chemical composition of mash mixtures.

Mash Mixture	Moisture per cent	Ash per cent	Protein per cent	Calcium per cent	Phosphorus per cent	Manganese per cent	Ca: P ratio
1	10.35	8.64	26.79	1.74	1.29	.0099	1.35:1
2	9.90	7.85	27.10	1.64	1.16	.0102	1.41:1
3	8.56	8.31	27.88	2.27	1.15	.0104	1.97:1

Table 3—Average body weights.

Group No.	Ration No.	Experiment One Day-old grams	Experiment One 4 weeks grams	Experiment One 4 weeks grams	Experiment Two Day-old grams	Experiment Two 4 weeks grams	Experiment Two 4 weeks grams
1	1	20.53	126.9	238.4	20.91	110.6	217.5
2	1	21.42	137.4	250.6	20.89	106.4	215.3
3	2	20.12	150.9	252.0	20.86	128.8	247.2
4	2	20.69	156.0	264.7	20.90	126.4	242.2
5	3	21.58	160.1	277.8	20.59	129.0	244.4
6	3	21.89	134.0	265.6	20.78	125.2	239.9
7	1s	21.59	154.4	257.5	20.78	115.1	227.7
8	1s	19.70	150.9	262.2	20.65	115.6	231.3
9	2s	21.00	161.8	270.6	20.66	118.0	233.7
10	2s	22.91	170.2	289.3	20.67	125.1	245.6
11	3s	22.58	158.4	266.4	20.62	118.5	231.4
12	3s	21.84	148.0	257.7	20.73	118.4	232.9

Table 4.—Group ranking on the basis of average body weights.

Group No.	Ration No.	Experiment One Day-old rank	Experiment One 4 weeks rank	Experiment One 6 weeks rank	Experiment Two Day-old rank	Experiment Two 4 weeks rank	Experiment Two 6 weeks rank
1	1	10	12	12	1	11	11
2	1	7	10	11	3	12	12
3	2	11	7	10	4	2	1
4	2	9	5	6	2	3	4
5	3	6	3	2	12	1	3
6	3	3	11	5	5	4	5
7	1s	5	6	9	6	10	10
8	1s	12	8	7	10	9	9
9	2s	8	2	3	9	8	6
10	2s	1	1	1	8	5	2
11	3s	2	4	4	11	6	8
12	3s	4	9	8	7	7	7

Table 5.—Weekly feed consumption per 100 chicks.

Experiment One

Group No.	Ration No.	Weeks of Age 1 pounds	2 pounds	3 pounds	4 pounds	5 pounds	6 pounds	Total pounds
1	1	6.05	16.67	31.28	42.74	45.65	58.41	200.80
2	1	6.64	18.66	27.68	40.38	47.53	55.69	196.58
3	2	7.03	13.38	24.06	40.00	47.49	53.10	185.06
4	2	7.27	14.34	25.00	40.33	48.98	54.47	190.39
5	3	3.96	16.17	24.44	25.38	43.66	40.54	154.15
6	3	4.48	15.03	24.26	26.31	43.34	41.66	155.08
7	1s	7.36	16.66	29.55	38.48	47.60	56.21	195.86
8	1s	7.25	16.95	29.03	39.86	48.17	56.36	197.62
9	2s	7.66	14.55	27.16	40.59	52.17	59.75	201.88
10	2s	8.23	12.90	24.72	37.50	47.75	51.49	182.59
11	3s	5.37	10.93	24.91	23.49	39.40	40.82	144.92
12	3s	5.58	9.49	27.45	21.76	41.92	37.37	143.57

Experiment Two

Group No.	Ration No.	Weeks of Age 1 pounds	2 pounds	3 pounds	4 pounds	5 pounds	6 pounds	Total pounds
1	1	7.50	17.15	21.63	31.64	41.51	52.57	172.00
2	1	7.09	17.64	26.60	32.13	46.20	63.77	193.43
3	2	7.08	17.71	22.89	32.06	39.41	52.85	172.00
4	2	7.50	15.82	21.00	31.99	41.65	49.21	167.17
5	3	5.83	14.70	16.59	24.36	33.74	41.51	136.73
6	3	5.42	14.63	16.03	26.88	35.49	38.15	136.60
7	1s	7.92	17.22	23.45	28.35	40.04	52.85	169.83
8	1s	8.36	16.73	23.59	29.82	43.40	55.79	177.69
9	2s	7.54	16.38	21.98	35.21	39.27	54.25	174.63
10	2s	7.08	16.24	21.35	33.39	40.32	51.10	169.48
11	3s	5.00	13.30	15.96	22.26	32.83	45.64	134.99
12	3s	5.44	12.18	16.52	21.56	32.62	45.08	133.40

Table 6.—Pounds of feed required to produce a pound of gain.

Group No.	Ration No.	Experiment One To 4 weeks pounds	Experiment One To 6 weeks pounds	Experiment Two To 4 weeks pounds	Experiment Two To 6 weeks pounds
1	1	4.13	4.18	3.94	3.97
2	1	3.65	3.91	4.43	4.51
3	2	2.93	3.62	3.35	3.45
4	2	2.91	3.54	3.28	3.43
5	3	2.29	2.73	2.57	2.77
6	3	2.84	2.89	2.73	2.83
7	1s	3.14	3.77	3.70	3.72
8	1s	3.22	3.70	3.75	3.83
9	2s	2.90	3.67	3.78	3.72
10	2s	2.57	3.11	3.39	3.42
11	3s	2.16	2.70	2.62	2.90
12	3s	2.31	2.76	2.59	2.85

Table 7.—Per cent mortality.

Experiment One (280 birds per group)

Group No.	Ration No.	Weeks of age 1 per cent	2 per cent	3 per cent	4 per cent	5 per cent	6 per cent	Total Recorded per cent	Lost per cent	Total Mortality per cent
1	1	9.64	1.43	0.00	0.00	1.79	0.36	13.21	5.00	18.21
2	1	1.07	0.36	0.00	0.00	1.43	0.00	2.86	2.50	5.36
3	2	1.79	1.07	1.07	0.36	0.36	0.71	5.36	2.50	7.86
4	2	5.36	0.71	1.07	0.71	0.00	0.00	7.86	3.93	11.79
5	3	2.50	0.36	0.00	0.00	0.00	0.00	2.86	2.86	5.72
6	3	1.07	0.36	0.71	0.36	1.07	0.00	3.57	1.43	5.00
7	1s	1.07	1.43	0.00	0.00	0.00	0.36	2.86	0.71	3.57
8	1s	2.50	1.79	0.00	0.00	0.36	0.00	4.64	3.21*	1.43
9	2s	6.78	2.50	0.00	0.36	0.00	0.00	9.64	8.57	18.21
10	2s	0.71	1.43	0.36	0.00	0.00	0.36	2.86	0.71	3.57
11	3s	0.71	1.43	0.71	0.00	0.00	0.36	3.21	2.14	5.36
12	3s	1.79	2.50	0.36	0.00	0.36	0.36	5.36	2.14	7.50

Experiment Two (240 birds per group)

Group No.	Ration No.	Weeks of age 1 per cent	2 per cent	3 per cent	4 per cent	5 per cent	6 per cent	Total Recorded per cent	Lost per cent	Total Mortality per cent
1	1	0.00	0.83	2.50	2.08	1.25	0.42	7.08	0.42	7.50
2	1	0.42	2.08	5.83	2.92	0.42	0.83	12.50	17.08	29.58
3	2	0.00	2.50	1.67	1.25	0.42	0.00	5.83	0.42	6.25
4	2	0.00	0.42	1.25	0.83	0.83	0.83	4.17	0.42*	3.75
5	3	0.00	1.25	0.83	0.42	0.00	0.00	2.50	0.00	2.50
6	3	0.00	0.83	0.83	0.83	0.00	0.42	2.92	0.42*	2.50
7	1s	0.00	1.67	1.67	5.00	0.42	0.42	9.17	1.25*	7.92
8	1s	0.42	0.00	1.25	7.08	1.25	0.42	10.42	0.42	10.83
9	2s	0.83	0.00	1.25	1.25	0.42	1.67	5.42	0.83*	4.58
10	2s	0.00	0.42	0.00	1.25	0.83	0.42	2.92	3.75*	0.83*
11	3s	0.00	0.00	2.08	1.25	0.00	0.00	3.33	5.00	8.33
12	3s	0.83	0.42	1.25	1.25	0.83	1.25	5.83	0.83*	5.00

*Extra birds in pen, from one of unbanded groups not in the experiments, birds weighed the same as those that lost their wing bands.

Table 8.—Cost of mash mixtures per bird and per pound of gain in body weight to 6 weeks of age.

(Costs based on prices of June 1, 1940)

Group No.	Ration No.	Experiment One Cost per bird cents	Experiment One Cost per pound of gain cents	Experiment Two Cost per bird cents	Experiment Two Cost per pound of gain cents
1	1	6.14	12.8	5.08	11.7
2	1	5.81	11.5	5.99	14.0
3	2	6.26	12.2	5.50	11.0
4	2	6.48	12.0	5.33	10.9
5	3	7.22	12.8	6.18	12.5
6	3	7.26	13.5	6.16	12.8
7	1s	5.94	11.4	5.03	11.0
8	1s	5.79	10.8	5.34	11.5
9	2s	7.07	12.8	5.59	11.9
10	2s	5.95	10.5	5.28	10.6
11	3s	6.88	12.8	6.88	13.7
12	3s	6.84	13.2	6.14	13.1

Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1941, **48**, 456-458

13355

Biotin and Prevention of Dermatitis in Turkey Poults.*

H. PATRICK, R. V. BOUCHER, R. ADAMS DUTCHER AND
H. C. KNANDEL.

From the Departments of Agricultural and Biological Chemistry and Poultry Husbandry, The Pennsylvania State College, State College, Pa.

During attempts to determine the riboflavin requirement of poults, in which both simplified and commercial type rations were used, dermatitis occurred regardless of the riboflavin intake. Jukes[1] reported that riboflavin prevented dermatitis in poults. Recently, Hegsted, *et al.*,[2] reported a dermatitis in chicks distinct from pantothenic acid deficiency and apparently unrelated to riboflavin. Since the dermatitis observed by us in turkeys appeared to be similar to that described by Hegsted, *et al.*, the essential rôle of biotin in turkey nutrition was investigated.

The basal ration had the following percentage composition: corn starch, 32.8; purified casein, 30; purified wheat bran, 20; polished rice, 5; soybean oil, 5; cod liver oil (400 D), 1; wheat germ oil, 2; mineral mixture, 4; choline, 0.2; thiamin, 0.0005. and pyridoxin, 0.0005. Day-old Bronze turkey poults were used throughout the experiment. They were kept in electrically heated brooders, equipped with hardware cloth floors. The experimental ration and water were always available.

The results recorded in Table I are typical of those secured in experiments involving more than 1000 poults. Incipient lesions appeared at 2 to 3 weeks of age. The areas between the toes, on the bottoms of the feet and at the proximal-dorsal portion of the upper beak showed definite lesions. The feet became completely encrusted and hemorrhagic cracks appeared as the manifestation of the deficiency progressed. The toes, in severe cases, became necrotic and sloughed off while the shank became calloused. The eyes became encrusted and eventually closed due apparently to an exudate. It is felt that this eye disorder is not necessarily an accompanying feature of the dermatitis since its severity did not parallel the incidence of dermatitis on the feet and beak.

---

*Authorized for publication on October 20, 1941, as paper No. 1052 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This research was aided by a grant from the Coöperative G. L. F. Exchange, Inc., Ithaca, N.Y.

[1] Jukes, T. H., *Poultry Sci.*, 1938, **17**, 227.

[2] Hegsted, D. Mark, Oleson, J. J., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1940, **20**, 599.

Crystalline riboflavin, varying from none to 0.0005%, did not protect the turkeys from dermatitis. Calcium pantothenate, nicotinic acid, cane molasses, the water-soluble fractions of dried beef liver and dried brewer's yeast, and the alcohol-soluble fraction of dried beef liver were also ineffective. The residues from liver and yeast gave complete protection.

TABLE I.
Dermatitis Preventive Action of Supplements.

Supplements			Results at 4 weeks of age		Disorders		
Riboflavin, %	Concentrates	Poults No.	Mortality %	Wt, g	Eyes %	Feet %	Beak %
0	None	6	83	82	83	34	83
.0002	,,	32	78	100	41	25	53
.0005	,,	18	72	203	22	44	67
,,	5% feeding cane molasses	21	71	181	48	43	62
,,	.014% Ca-pantothenate	18	39	210	55	78	83
,,	.4% nicotinic acid	12	58	115	0	50	50
,,	8% filtrate from peptic digest of liver	12	42	190	0	0	0
,,	10% norite adsorbate of filtrate from peptic digest of liver	12	87	202	0	0	0
,,	5% water extr. of liver (4080)*	6	100	—	33	100	100
,,	4% alcohol extr. of liver (4303)*	6	17	296	0	33	50
,,	10% liver residue (4081)*	5	0	392	0	0	0
,,	10% desiccated liver	8	0	417	0	0	0
,,	3.2% R-S concentrate†	12	87	200	25	75	75
,,	5% yeast residue†	26	4	330	0	0	0
	5% ,, ,,	22	36	136	0	0	0
,,	5% dried yeast‡	20	5	381	0	0	0
,,	40 rat units biotin daily per poult	10	10	180§	0	0	0
,,	Control for biotin supplemented group	9	44	182§	11	89	89

*Prepared by methods described by Hogan, A. G., Richardson, L. R., Patrick, H., and Kempster, H. L., *J. Nutr.*, 1941, **21**, 327.

†Prepared as outlined by Schumacher, A. E., and Heuser, G. F., *Poultry Sci.*, 1940, **29**, 315.

‡Strain-S brewer's yeast generously furnished through the courtesy of Dr. K. L. Cartwright of Anheuser-Busch & Co.

§These body weights were taken at 21 days.

György, *et al.*,[3] stated that biotin could not be removed from liver or yeast by ordinary methods of solvent extraction but could be removed by peptic digestion. Accordingly liver was digested with pepsin and the filtrate concentrated *in vacuo*. The peptic filtrate and a norite adsorbate of the peptic filtrate were both active. This suggested that biotin was probably the active factor present in the liver and yeast residues. To confirm this point a biotin concentrate (S.M.A. 5000) reportedly free of other members of the B complex, was fed daily by pipette at a level of 40 rat units per day. Complete protection from dermatitis resulted while a group receiving the same ration, but without biotin, developed severe dermatitis in 89% of the poults.

Poults receiving the basal ration supplemented with 5% yeast residue grew slowly and did not develop dermatitis. Poults receiving the basal ration plus 5% yeast residue and 0.0005% riboflavin grew rapidly without developing dermatitis.

Under the conditions of this experiment riboflavin did not prevent dermatitis in turkey poults, while biotin at a level of 40 rat units per day was effective. Other concentrates used in feeding trials, reported in Table I, also possessed the dermatitis-preventing properties ascribed to biotin.

---

[3] György, P., Kuhn, R., and Lederer, E., *J. Biol. Chem.*, 1939, **131**, 745.

Observations on the Pathology of Dairy Calves on Low Vitamin A Diets

W. T. S. THORP, D.V.M., M.S., H. A. KEENER, Ph.D.,
S. I. BECHDEL, Ph.D., and N. B. GUERRANT, Ph.D.
State College, Pa.

Reprint from American Journal of Veterinary Research, Vol. III, No. 6,
January, 1942, pp. 27-31.

Observations on the Pathology of Dairy Calves on Low Vitamin A Diets*

W. T. S. THORP, D.V.M., M.S., H. A. KEENER, Ph.D., S. I. BECHDEL, Ph.D., and N. B. GUERRANT, Ph.D.

State College, Pa.

ABNORMAL, PHYSIOLOGIC and tissue changes due to a low intake of carotene have been reported in the dairy calf.

REVIEW OF LITERATURE

Moore, Huffman and Duncan[1] reported on a blindness in calves of nutritional origin associated with constriction of the optic foramen. Moore[2] reported that calves fed low carotene rations developed nyctalopia, papilledema and a permanent blindness due to constriction of the optic nerve. Guilbert and Hart[3] established the relationship of night blindness to vitamin A deficiency in the bovine species. A degeneration of the germinal epithelium of the testes, absence of spermatozoa in the epididymus and changes in the pituitary gland were reported by Sutton, Krauss and Hansard.[4] Moore and Sykes[5] showed that increased cerebrospinal pressure was associated with vitamin A-deficient diets in the bovine species. This observation was found to correlate with the papilledema occurring in calves. Langham, Sholl and Hallman[6] described a degeneration of the tubular epithelium, numerous lesions of cellular infiltration and proliferation and fibrosis in the kidney of cattle deficient in vitamin A.

From the above, one would conclude that certain physiologic tissue changes are associated with a low intake of vitamin A.

MATERIALS AND METHODS

During the past 2½ years, 25 calves from the dairy experimental herd were autopsied in the course of vitamin A studies.

These 25 cases were studied grossly and microscopically and are divided into three different groups. The animals of each group received different amounts of vitamin A in the form of carotene. Group 1 consisted of 13 calves which had received the lowest level of carotene, with an average daily intake of 6.7 micrograms per calf. Group 2 consisted of eight calves which had received a low to medium intake of carotene, with an average of 15.8 micrograms per calf daily. Group 3 consisted of four calves which had received the highest levels, or an average daily intake of 19.7 micrograms. Detailed data concerning the blood carotene, etc., are given elsewhere.[7]

Per Pound Bodyweight

GROSS PATHOLOGY

Enteritis was observed in eleven of the cases in group 1, five of the seven in group

---

*Paper No. 1026, journal series, Pennsylvania Agricultural Experiment Station.

[1] Moore, L. A., Huffman, C. F.. and Duncan, C. W.: Blindness in cattle associated with a constriction of the optic nerve and probably of nutritional origin. J. Nutri., ix (1935), p. 533.

[2] Moore, L. A.: Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and nyctalopia in calves. J. Nutri., xvii (1939).

[3] Guilbert, H. R., and Hart, G. H.: Minimum vitamin A requirements with particular reference to cattle. J. Nutri., x (1935), p. 409.

[4] Sutton, T. S., Krauss, W. E., and Hansard. S. L.: The effects of vitamin A deficiency on the young male bovine. Abst., J. Dairy Sci., xxiii (June 1940); Sutton, T. S., and Brief, B. J.: Anatomical and physiological changes in the pituitary glands of vitamin A deficient rats. Bul. 610, Ohio Agr. Exp. Sta.

[5] Moore, L. A., and Sykes, J. F.: Cerebrospinal fluid pressure and vitamin A deficiency. J. Physiol., cxxx, p. 684.

[6] Langham, R. F., Sholl, L. B., and Hallman, E. T.: The pathology of the bovine kidney in vitamin A deficiency. Abst., J.A.V.M.A., xcvii (Nov. 1940), pp. 542-543.

[7] Unpublished data from the Dept. of Dairy Husbandry, Pennsylvania State College.

2 and two of the four in group 3. The prevalence and severity of the enteritis appeared greatest in the calves on the lowest levels of vitamin A. Diarrhea, which was observed periodically during the course of the study, became more severe toward the end of the experiment. As a rule, the chyme consisted of some food and large quantities of mucus. Upon section, the mucosa was found to be swollen and thickened and the mesenteric lymph nodes were swollen and edematous.

The gross lesions in the kidneys varied in individual cases. The predominate lesions were numerous white foci ranging in size from pin point to 1 mm. The capsules stripped easily from the cortex with the exception of two atypical cases in group 1. These two were typical of "white-spotted kidney" in cattle. Upon section some of the pin-point foci were found to extend into the cortex while others noted were deeper in the cortical substance. With the exception of the two cases mentioned above, lesions were found in ten of the cases in group 1, one of the eight in group 2 and one of the four in group 3.

Grossly the liver lesions were not numerous and they varied considerably. A mottling of the parenchyma of the liver suggesting necrosis was the predominate change. However, one case in group 2 had multiple abscesses of long duration, while two in group 1 suggesting fibrosis of the liver were the exceptions. In three cases, the gall bladder contained considerable dark, thick bile. Eight of the cases in group 1 showed liver lesions, two in group 2 and one in group 3.

A typical bronchopneumonia was observed in 4 of the 25 cases. Two of them were on the lowest level of vitamin A. The others were on the next to the lowest level, or group 2.

HISTOPATHOLOGY

Sections for microscopic study were fixed in both Zenker's solution and formalin. In a few cases Bouin's solution was used. Sections were stained with Heidenhain's aniline blue, Verhoeff's elastic tissue stain, sudan IV for fat and eosin hematoxylin.

The most conspicuous lesions were observed in the kidney, liver, intestine and testicle.

Degeneration of the epithelium of the proximal convoluted tubules existed in all of the groups, but it was most extensive in group 1. The degeneration and necrosis in Henle's loop and the distal convoluted tubules were not as conspicuous as in the other parts of the tubules. Two cases in group 1 showed some evidence of fatty degeneration of the epithelium in the proximal convoluted tubule. Hyaline-like material in the tubular epithelium was present in a number of cases. This was especially noticeable in those areas where considerable atrophy and degeneration of the tubules had taken place. Those areas which contained grayish-white pin-point foci were characterized by accumulations of lymphocytes and macrophages (figs. 1 and 2). In other areas there was a marked proliferation of fibroblasts and considerable fibrosis (fig. 3). In some of these areas a complete degeneration of the tubules had occurred. In the more conspicuous lesions there was a connective tissue thickening of

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE—FIGS. 1-8

1) Section through the cortex of the kidney, showing one of the pin-point lesions noted in gross. Note the loss of the tubules and accumulation of lymphocytes and macrophages (x 85).

2) Section of the cortex, showing atrophy of the tubules and a marked cellular infiltration (x 85).

3) Section of the cortex, showing atrophy of the tubules and early fibrosis (x 85).

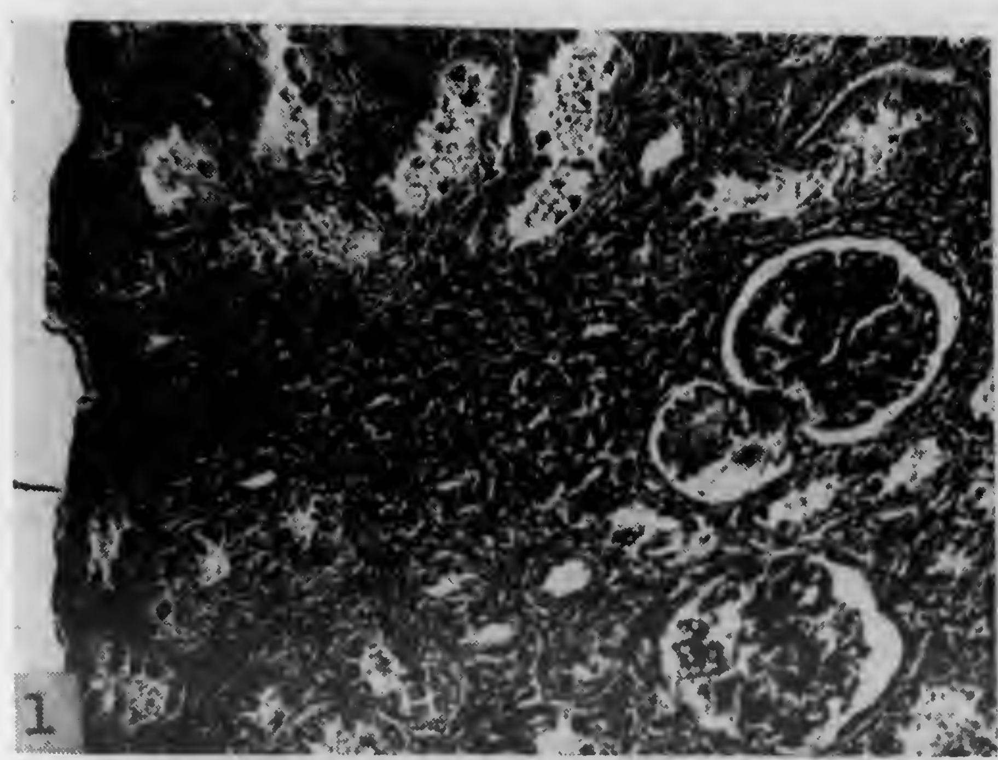
4) Section at the periphery of the renal corpuscle, showing early fibrosis of Bowman's capsule (x 455).

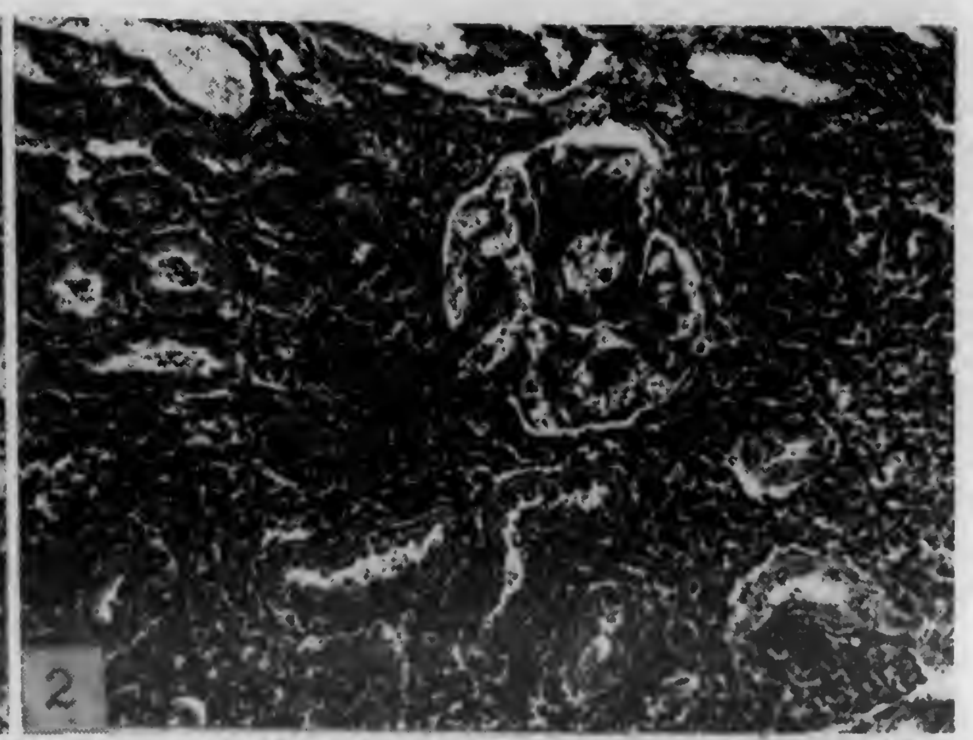
5) Section, showing marked thickening and hyalinization of Bowman's capsule (x 450).

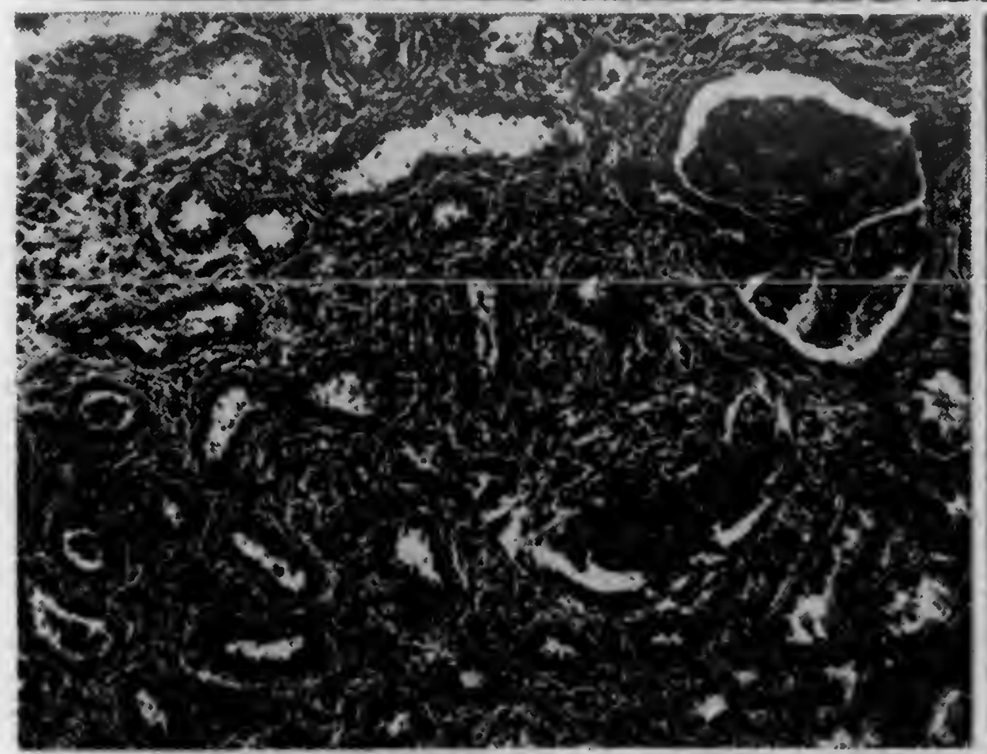
6) Section of the cortex, showing recent interstitial infiltration of lymphocytes and macrophages with marked hemorrhage into the tubules (x 465).

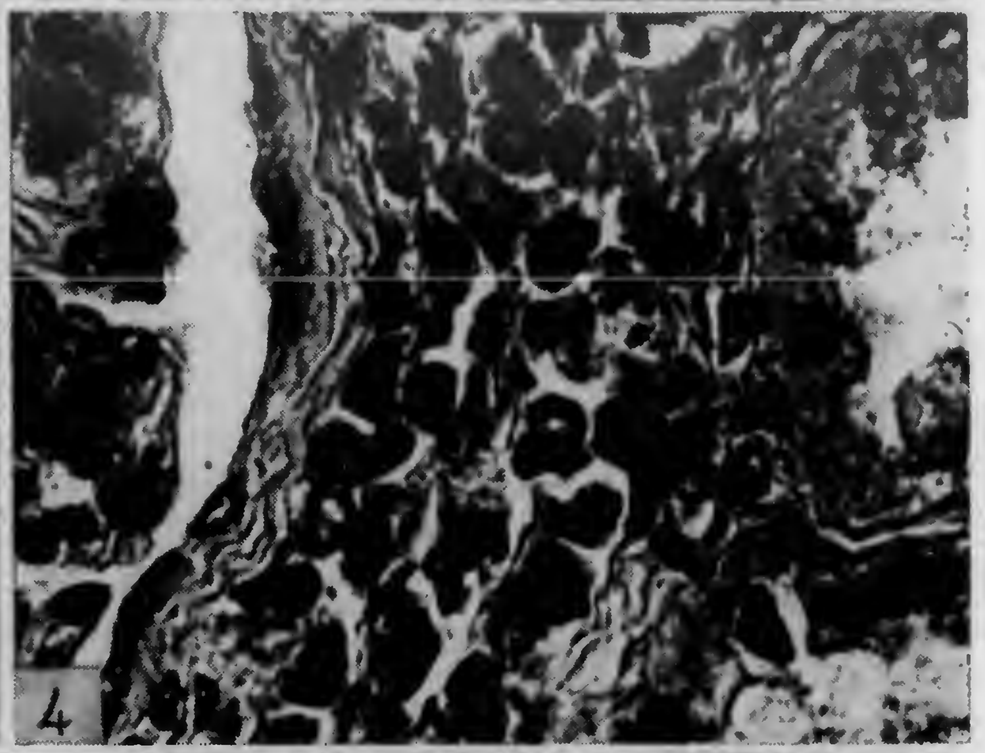
7) Section, showing an infiltration of lymphocytes and macrophages about the tubules with early degeneration of the tubular epithelium (x 450).

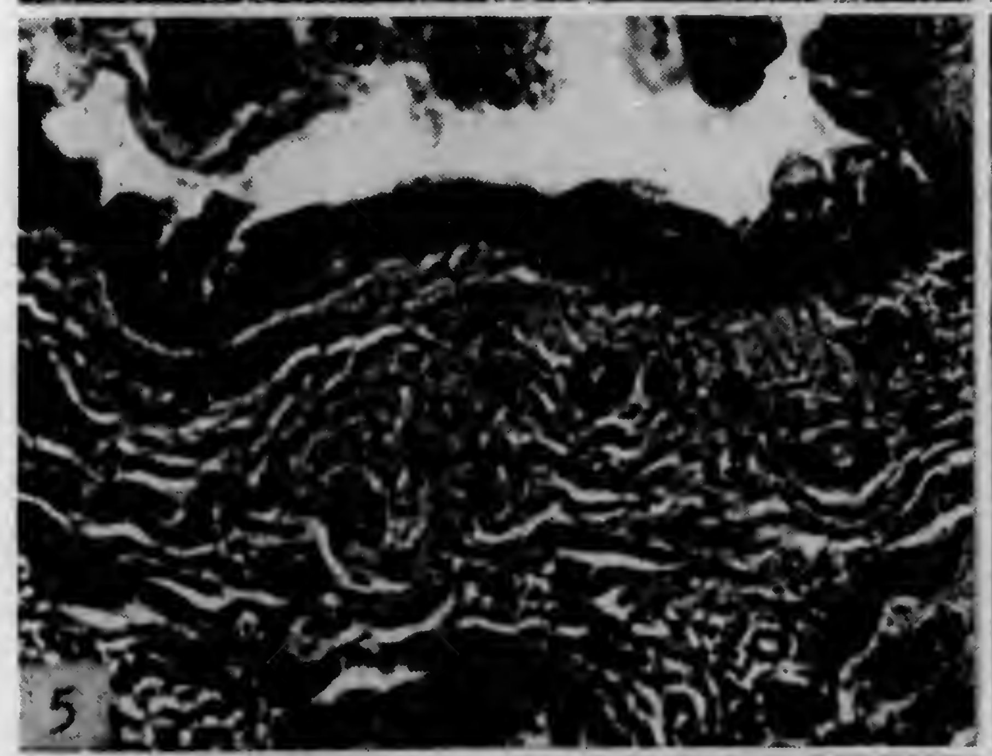
8) Section of small intestine, showing necrosis and inflammatory changes with some thickening of the mucosa (x 85).

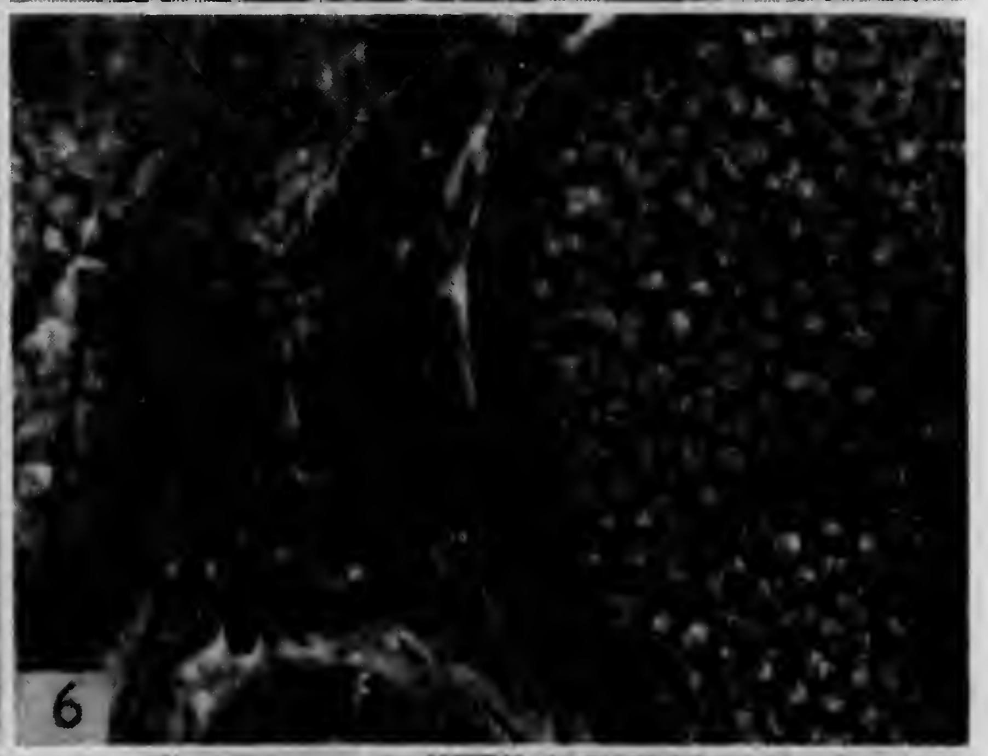


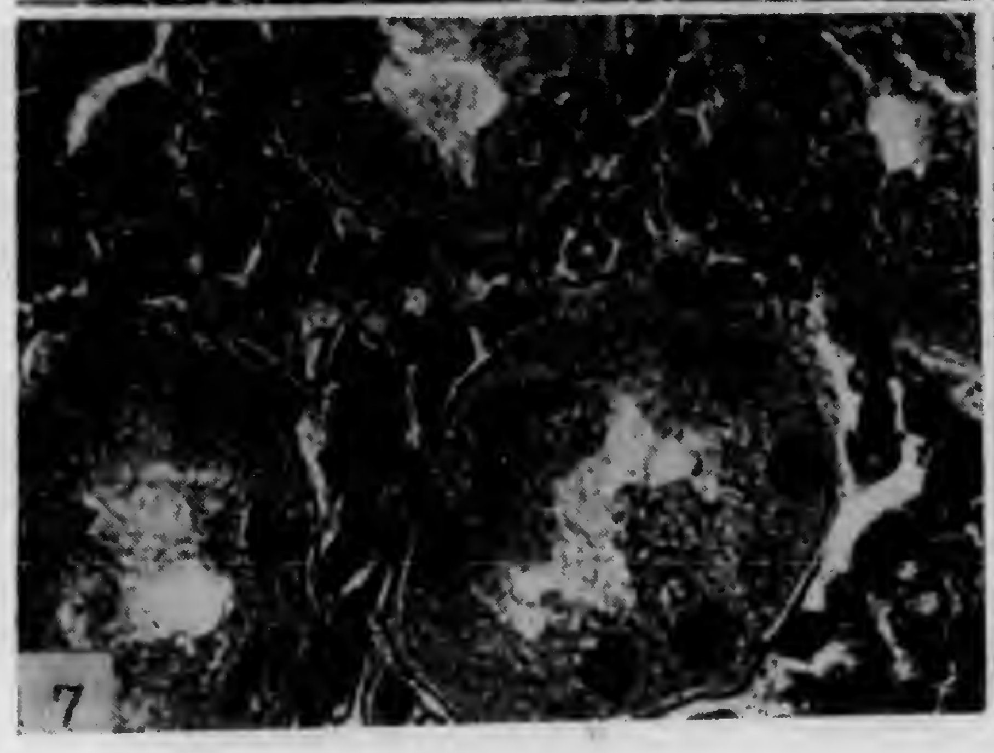


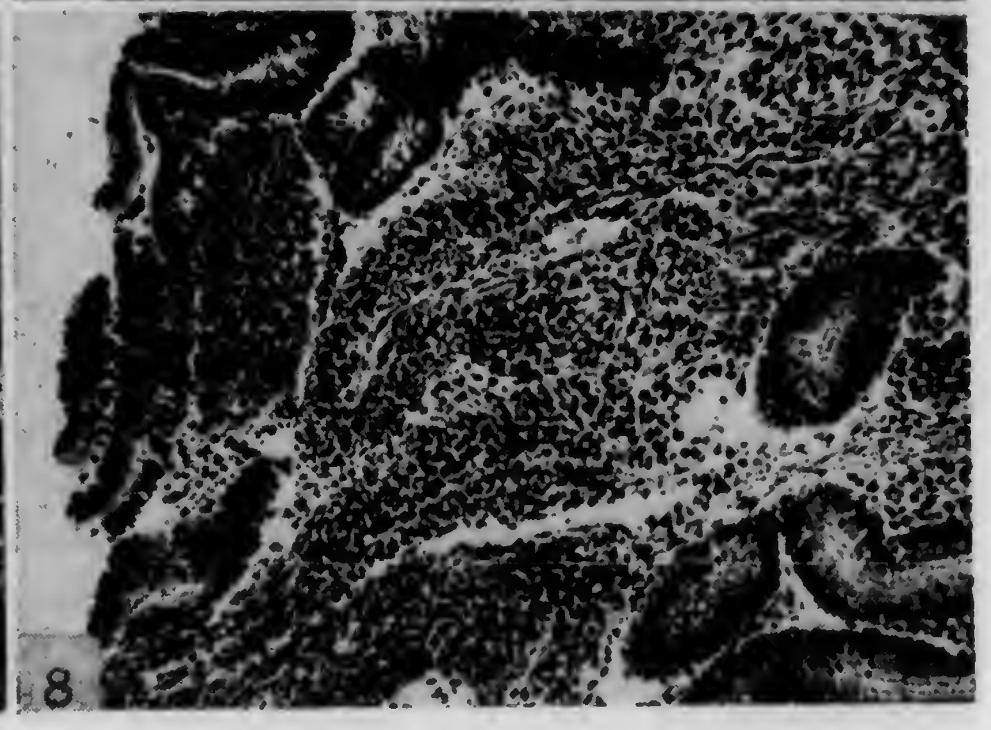


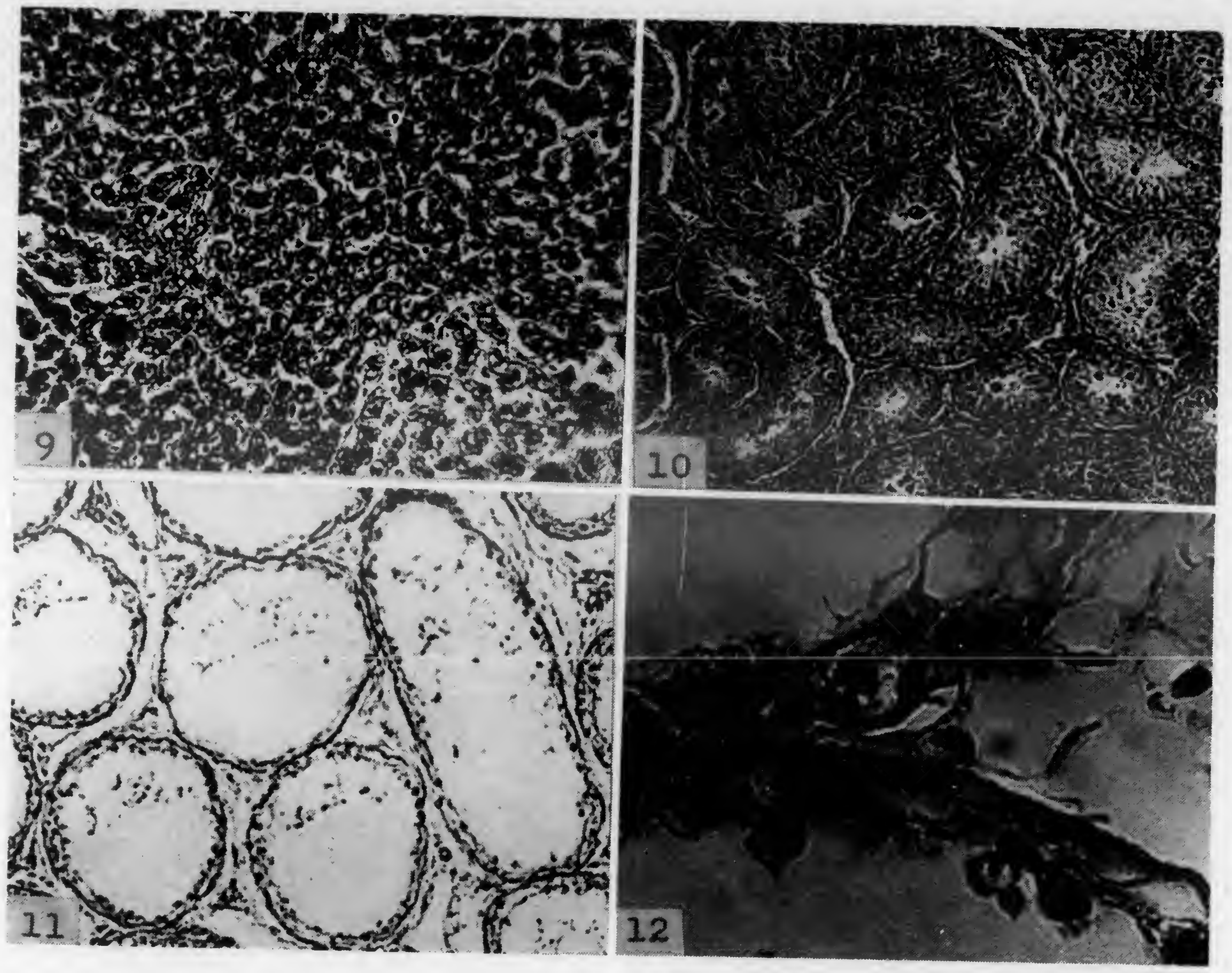












FIGS. 9-12

9) Section of liver, showing degeneration of the cells (x 70).

10) Section of normal, developing testicle (x 115).

11) Section of testicle, showing extensive degeneration of the germinal epithelium (x 120).

12) Section of testicle, showing degenerated germinal epithelial cells (x 475).

Bowman's capsule (figs. 4 and 5) accompanied by some atrophy of the glomerular tufts. Considerable fibrosis was observed in the tissues adjacent to the atrophied and thickened renal corpuscle. With but two exceptions the cases showing the most fibrosis and degenerative changes were in group 1. In these two cases there was a moderate degeneration of the tubular epithelium and early cellular infiltration with very little proliferation of fibroblasts. In three cases of group 1, considerable hemorrhage into the tubules was noted (fig. 6). The lesions in which there was an infiltration of lymphocytes and macrophages about the tubules were usually accompanied by an early degeneration of the tubular epithelium (fig. 7).

The intestinal mucosa of most of the calves showed a varying degree of morbidity. Most of the lesions consisted of a mild, somewhat hyperplastic enteritis beginning in the upper small intestine and extending to the ileocecal valve. Considerable necrosis and desquamation of the epithelium were present in some cases (fig. 8). Sections removed at 4- to 6-ft. intervals beginning just below the pylorus seemed to indicate an increase in the severity of the lesions in passing toward the ileocecal valve. Some necrosis of the epithelium and infiltration of the stroma of the glands with lymphocytes and macrophages were noted, accompanied in some areas by the proliferation of fibroblasts. A large number of eosinophiles were present in the mucosa. With the exception of three cases that showed an acute diarrhea clinically, there was no evidence of desquamation and ulceration of the mucosa.

Microscopically the most consistent lesions seen in the liver were cloudy swelling and necrosis, and in five cases of group 1 there was some fatty degeneration (fig. 9). In the cases where necrosis of the liver cells had taken place, there were small foci of macrophages and lymphocytes.

The lesions in the testicle consisted of a degeneration and necrosis of the germinal epithelium lining the tubules (figs. 10 and 11). No marked interstitial changes were observed. There were sporadic attempts at spermatogenesis in these areas. Areas in which the epithelium was not entirely destroyed showed many pyknotic and degenerate nuclei (fig. 12).

Metaplasia of the epithelium was noted to some extent in the calices of the kidney, and in two cases this was observed to a moderate degree in the trachea. These changes were not as conspicuous in the calves on the lower levels as one would expect.

DISCUSSION

Although some variations occurred in the microscopic pathology of the tissues studied, certain degenerative and inflammatory lesions occurred consistently. With the exception of the lesions observed in the liver tissue, changes were more extensive in those cases on the lowest levels of carotene. The duration, severity and extent of the lesions and symptoms in the intestinal tract were manifested in the liver and kidney. This was especially true as regards the kidney lesions. Although the enteritis in most cases was a mild, somewhat hyperplastic lesion, a correlation appeared to exist between the extent of the intestinal pathology and the pathology of the other tissues. The liver lesions were the most variable. In three of the cases on the lowest levels of vitamin A, there were no marked liver lesions although considerable diarrhea, enteritis and conspicuous kidney lesions existed.

The degenerative changes in the testicle did not exist in the cases on the higher levels of vitamin A. A number of the cases were autopsied too young to determine the effect on the number of spermatozoa, but degenerative changes were observed in the germinal epithelium which probably would have affected spermatogenesis in 9 of the 13 cases in group 1.

In considering all the lesions found on the three levels of carotene, it is obvious that the more marked lesions occurred in the lowest level of vitamin A. This difference in the various groups was noted especially in the pathology of the kidney.

SUMMARY

1) Twenty-five calves fed three different suboptimal levels of carotene were studied grossly and microscopically for pathologic changes.

2) Degenerative and inflammatory changes were observed in the kidneys of calves on the lower levels of vitamin A.

3) An enteritis with some thickening of the mucosa accompanied by a persistent diarrhea was observed.

4) Degeneration and necrosis were present to a variable extent in the liver.

5) Degenerative changes also were observed in the testicle. These probably inhibited normal spermatogenesis.

6) Metaplasia of the epithelial tissues was not consistently observed.

7) A correlation appeared to exist between the lesions in the intestines and in the kidneys, both in extent and severity. In a limited number of cases the liver could be included in this relationship.

8) In general, the lesions observed were more conspicuous in the calves which had received the lowest levels of vitamin A.

Sulfapyridine in the Treatment of Calf Pneumonia

W. T. S. THORP, D.V.M., J. F. SHIGLEY, B.Pd., D.V.M., and
A. K. ANDERSON, Ph.D.
State College, Pa.

Reprint from Journal of the American Veterinary Medical Association, Vol. C, No. 780,
March 1942, pp. 225-228.

Sulfapyridine in the Treatment of Calf Pneumonia*

W. T. S. THORP, D.V.M., M.S.; J. F. SHIGLEY, B.Pd., D.V.M.; and A. K. ANDERSON, Ph.D.
State College, Pa.

THE MORBIDITY and mortality of calf pneumonia is highest in calves ranging in age from 2 to 10 weeks. In a large percentage of cases it is a sequel to calf scours. Calf pneumonia usually comes on suddenly, first manifesting itself by a dry cough, extreme lacrimation, a rise in temperature (104.8 to 105.5 F.), and usually a high blood leukocyte count. Manifestations of a bronchopneumonia soon follow the above symptoms. Carpenter and Gilman[1] described the lesions briefly and suggested streptococci, staphylococci and coliform organisms as possible causative agents. The tissue changes have been described by Thorp and Hallman[2] which are briefly a subacute or chronic bronchitis associated with consolidation of the lungs which begins in the anterior lobes and progresses posteriorly involving a variable portion of the diaphragmatic lobes.

Calf pneumonia is a serious problem in many large dairy herds in widely separated portions of the country. Sanders[3] describes the condition in Florida and attributes it to a weakened condition of the calf due to parasites and an adverse environment, which is followed by an infection with *Pasteurella bovisepticus*. Recent work at this station suggests either a pasteurella organism or some form of the hemophilus group of organisms[4].

The cases considered here are from one large dairy herd in which 15 per cent to 20 per cent losses from calf pneumonia had occurred during the winter and spring months. Twenty-five per cent of the cases of pneumonia followed calf scours.

The use of sulfapyridine in calves has been reported by Ehlers[5] in calf septicemia. It has been used successfully by Farquharson[6] in the treatment of calf diphtheria. A study of the administration of sulfapyridine to the mature bovine has been reported by Klein, Kleckner and Biltz[7].

With the above reports in mind and other uses to which sulfapyridine has been put an attempt was made to use it in the treatment of calf pneumonia during the past winter and spring.

SULFAPYRIDINE THERAPY

All calves in this herd which showed typical symptoms of pneumonia were hospitalized and sulfapyridine treatment started. Table I gives a summary of the 16 calves treated. The dosage was determined on the basis of weight and varied somewhat in individual cases. However, the initial dose ranged from 0.05 to 0.1 Gm. per lb. of body weight. In some animals the total daily dose was divided into two doses administered morning and night while in others the total dose was divided and given three times per day about six hours apart.

In calves 11 and 16, the initial dose for the first two days was high and then reduced for the remaining three days of treatment. The response in these calves was the best of any treated. In calf 13, there was no response to the sulfapyridine

---

*Authorized for publication on September 9, 1941 as paper No. 1048 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[1] Carpenter, C. M. and Gilman, H. L., Studies in Pneumonia in Calves. Cornell Vet. xi (1921), p. 111.

[2] Thorp, W. T. S. and Hallman, E. T., Pathology of Calf Pneumonia. J.A.V.M.A., xciv, Part II (April 1939) p. 365.

[3] Sanders, D. A., Enzootic Bronchopneumonia of Dairy Calves. Tech. Bulletin 346, Florida Agr. Exp. Sta., June 1940.

[4] "Studies on the Etiology and Pathology of Calf Pneumonia." Unpublished data presented at the Research Section, 78th annual A.V.M.A. convention, Aug. 13, 1941.

[5] Ehlers, D. C., Sulfapyridine in Calf Septicemia. Veterinary Medicine, xxxv (June 1940), p. 329.

[6] Farquharson, James, Sulfapyridine in the Treatment of Calf Diphtheria. J.A.V.M.A., xcvii (1940), p. 431.

[7] Klein, L. A., Kleckner, A. L., and Biltz, R. O. Sulfapyridine in Cattle: a Contribution to Its Pharmacology. Presented at 77th Annual Meeting, Abstract J.A.V.M.A., xcvii (Nov. 1940), p. 544.

TABLE I—Summary of Sixteen Cases of Calf Pneumonia Treated with Sulfapyridine

CALF NO.	AGE IN WEEKS	WEIGHT LBS.	INITIAL TEMP. °F.	TEMP. IN 24 HRS. °F.	INITIAL LEUKOCYTE COUNT	LEUKOCYTE COUNT AFTER TREAT-MENT	TOTAL DAILY DOSAGE, GMS. DAYS 1ST	2ND	3RD	4TH	5TH	6TH	NO. OF DOSES* PER DAY	RESULTS
1	4	75	104.2	101.8			5.	5.	5.	2.	1.		2**	Recovery
2	3	80	103.8	101.5			5.	5.	5.				2	Recovery
3	1	60	103.2	101			5.	5.	5.	2.	2.		2	Slow Recovery
4	4	80	103.8	102.2			5.	5.	5.				2	Fair Recovery
5	2	70	104	102			5.	5.	5.				2	Fair Recovery
6	4	70	103.8	101			5.	5.	5.				2	Slow Recovery
7	5	75	105	102	12,500	7,800	5.	5.	5.	2.	1.		3	Good Recovery
8	3	70	104.2	103	11,500	7,600	5.	5.	5.	2.	2.		3	Good Recovery
9	2	80	104.1	102.8	15,800	8,000	5.	5.	3.	2.	2.		2	Recovery
10	4	82	104.5	103.2	16,000	8,200	5.	5.	5.	2.	2.	2.	2	Recovery
11	1	85	102.8	101	14,200	7,900	7.5	5.	5.	2.	1.		1st da. 3 other 2	Good Recovery
12	2	90	104.2	102	15,200	9,000	7.5	7.5	5.	2.	1.		1st da. 3 2nd da. 3 3rd da. 2	Good Recovery
13	3	90	104	102.5	17,000	17,500	7.5	7.5	5.	2.	1.		Same as No. 12	Died
14	2	95	103.8	101.8	15,000	8,500	7.5	5.	5.	1.			Same as No. 12	Good Recovery
15	3	90	104.5	103	14,500	2,600	5.	5.	2.	1.			2	Died
16	3	80	104.5	102	18,800	10,000	7.5	7.5	5.	5.	1.	1.	3	Good Recovery

*All doses given orally as a drench
**Division of total daily dosage

whatever with practically the same treatment as calves 11, 12 and 16. At the autopsy of calf 13, a subacute pneumonia was observed in the anterior lobes of both lungs with about one-half of the diaphragmatic lobes showing an acute pneumonia. There was, in addition to the pneumonia, an extensive nephritis associated with numerous congenital cysts in the kidneys. The nephritis and the congenital cysts probably accounted in part for the lack of response to the treatment. This calf had received a three-day treatment of sulfapyridine five days previous to the treatment reported here. Severe scours preceded the pneumonia treated in this case. In the case of calf 15, the pneumonia followed a severe attack of scours and upon autopsy the typical lesions of an acute bronchopneumonia were observed. A severe nephritis was noted with a small number of urinary calculi in the pelvis of the kidney. Upon microscopic study of the tissues, subacute to chronic inflammatory changes were observed in the interstitial tissues of the kidney. Moderate degeneration of the tubular structures was observed which probably was the result of the severe scours preceding the pneumonia. The lowering of the erythrocyte count to 3,200,000 and the neutropenia observed during the last two days of treatment were characteristic of the toxic symptoms described in other subjects.

EXPERIMENTAL CALVES

Of the eight calves given sulfapyridine, two were acute cases of pneumonia and six were normal calves treated in an effort to study the relation of dosage to the blood level of sulfapyridine for different ages and weights. All the total daily dosages were divided into three equal doses and given approximately every six hours. The free sulfapyridine in the blood was determined by the method described by Bratton and Marshall.[8]

A summary of the data for this group of calves is given in table II. Calves 2 and 3 which were diagnosed as acute calf pneumonia responded very well to the treatment which produced an average blood level of 4.12 mgs. per cent for calf 2 during the first four days and 5.75 mgs. per cent for calf 3 over the same period. The initial dose in the latter was the highest. Calves 1, 4 and 5 are probably the most ideal blood levels to maintain.

In these three calves, a large initial dose was given for the first two days and then gradually reduced. Calves 6, 7 and 8 were given large doses to determine the upper limits of the blood level for sulfapyridine. In all of these calves, toxic symptoms were observed after two days of treatment. It will be noted from table II that in all of these cases the average blood level of sulfapyridine is 10 mgs. per cent or above. Toxic symptoms were not pronounced in calves 6 and 7, but microscopic examination of the blood revealed some anemia and a rise in the blood leukocyte count. The most pronounced symptoms were observed in calf 8, which upon autopsy showed numerous calculi in the pelvis of the kidney and many yellowish white streaks in the pyramids. Microscopically, sections of the kidney showed some hemorrhage into the tubules and a few tubules contained a small number of casts.

DISCUSSION

The response to treatment with sulfapyridine differed in individual calves; however, certain consistent changes were observed. Calves receiving a large initial dose followed by a subsequent reduction of the dosage made the most rapid recovery. Calves 13 and 15 of table I would indicate that in cases where other pathology was present, sulfapyridine would be contraindicated.

On the basis of the two groups of calves it is obvious that age difference along with body weight is an important factor in regard to the calf's tolerance to the administration of sulfapyridine. In the younger calf large dosages should not be continued for any length of time. Deter-

---

[8] Bratton, A. C., and Marshall, E. K.: Jl. Biol. Chem., cxxviii (1939), p. 537.

TABLE II—Blood Determinations on Eight Experimental Calves

CALF NO.	AGE	WT. LBS.	TOTAL DAILY DOSAGE, GMS DAYS 1ST	2ND	3RD	4TH	5TH	6TH	MGS. % OF FREE SULFAPYRIDINE IN THE BLOOD DAYS 1ST	2ND	3RD	4TH	5TH	6TH	TIME SAMPLE WAS DRAWN	REMARKS
1	2 weeks	95	7.5	7.5	5.	5.	2.	2.	2.30 7.24	7.38 7.78	9.66	7.55	6.06	2.80	9 AM 3 PM	Normal
2*	2 weeks	75	5.	5.	5.	2.	1.		4.65	3.49	5.32	3.03	2.60		9 AM 1 PM	Recovered
3*	6 days	80	7.5	5.	2.	2.	1.		5.00	6.35	7.64	4.76	1.42		9 AM 1 PM	Recovered
4	2 weeks	102	7.5	7.5	5.	5.	2.	2.	6.35 9.63	7.28 8.17	8.69 6.78	9.65 10.41	4.68 4.12	2.31 2.42	1 PM 9 PM	Normal
5	5 weeks	150	7.5	7.5	5.	5.	2.2	2.	3.55 7.78	6.39 8.27	7.50 6.92	5.19 5.87	4.62 3.74	2.39 2.22	1 PM 9 PM	Normal
6	4 weeks	130	12	12	4.	4.	3.	3.	2.07 6.05	8.20 9.39	7.75 15.74	12.90 14.12	10.58 8.93	4.26 3.81	1 PM 9 PM	Slight evidence of Toxicity
7	8 days	95	7.5	7.5	5.	5.	2.	2.	6.72 10.52	6.34 10.45	10.69 9.55	4.79 9.05	5.41 5.71	3.55 2.93	1 PM 9 PM	Toxic Symptoms
8	5 days	75	7.5	7.5	5.	5.	2.	2.	1.30 4.08 9.39 10.73	9.31 10.80 14.19 11.15	13.96 16.00 13.46 16.05	15.62 12.55 13.32 12.28	20.62 21.74 Died		9 AM 1 PM 5 PM 9 PM	Died

*Clinical symptoms of acute calf pneumonia minations of sulfapyridine in the blood of the experimental calves indicate that the blood level of sulfapyridine should not exceed 10 mgs. per cent. The most desired level appears to fall between 5 and 8 mgs. per cent. In the younger and smaller calves, 5 and 6 mgs. per cent is probably sufficient while in larger and older calves a slightly higher level would probably give better results. The level of sulfapyridine in the blood of calves is not proportional to the dosage per pound of body weight. On the basis of the two groups of calves administered sulfapyridine in the course of this work the following daily dosages can be recommended. In calves up to 70 lb., 0.05 Gm. per lb. of body weight, calves 70 to 100 lbs. 0.06 Gm. per lb. of body weight, and over 100 lbs., 0.07 Gm. per lb. of body weight. These dosages are higher than that recommended by Farquharson for the treatment of calf diphtheria; however, no intravenous dose was given as the initial dosage. Higher dosages were given without severe symptoms of toxicity appearing. On the basis of the cases treated, a reduction of 0.02 Gm. per lb. of body weight every two days is deemed advisable since there is some evidence that the drug may be accumulative. The dosage should be given three times daily and in proportion to age and weight.

SUMMARY

1. Twenty-four calves were given sulfapyridine, 18 of which had symptoms of acute bronchopneumonia.

2. Blood determinations were made on eight of the calves given sulfapyridine. These results indicate that the blood level should not exceed 10 mgs. per cent.

3. A significant rise in the leukocyte count, followed by a neutropenia and lowering of the erythrocyte count, were observed in the cases where toxic symptoms occurred.

4. Younger calves were more subject to symptoms of toxicity. The following initial dosages are suggested: Calves up to 70 lb., 0.05 of Gm. per lb. of body weight; calves 70 to 100 lb., 0.06 of Gm.; and calves over 100 lbs., 0.07 Gm. per lb. of body weight.

5. It is realized by the authors that blood determinations were only made on eight of the calves and that the total number treated is small. Caution should be observed in the use of the drug, but it shows promise of being a fairly reliable treatment for calf pneumonia.

ACKNOWLEDGMENTS

The writers wish to thank Professor A. A. Borland and Mr. P. D. Jones of the Department of Dairy Husbandry for providing certain facilities and experimental calves for this work and to Mr. Frank Singer of the Department of Agricultural and Biological Chemistry for assistance in the blood determinations, and to E. R. Squibb and Sons, New York, for their coöperation in providing the sulfapyridine.

BULLETIN 422 MARCH 1942

BACTERIAL LEAFSPOTS OF PENNSYLVANIA TOBACCO

I. Occurrence and nature of the microorganism associated with wildfire

TOBACCO SEEDLINGS IN LANCASTER COUNTY

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

CONTENTS

PAGE

Bacteriological Methods 2

Analysis of soil samples—Analyses of plant material—Cultures employed—Morphological and physiological characteristics—Single-cell isolations—Serological techniques — Preparation of antisera — Agglutination technique—Agglutinin absorption—Production of Dawson "S" forms.

Experimental Results Showing Nature of Organism... 5

Morphological and physiological characteristics of green fluorescent isolates — Serological studies — Serological studies employing single-celled cultures—Antigenic relation of decapsulated green fluorescent bacteria—Variation in antigenicity of green fluorescent bacteria.

Discussion and Application of Results............... 25

Practical value of the antigenic identity found to exist between *Ph. tabaci* and *Ps. fluorescens*—Influence of steaming on microorganisms in tobacco seedbeds—Increase in bacterial numbers following steaming—Bacterial flora of the tobacco plant.

Antigenic Relationships 33

Summary 34

Bibliography 35

BACTERIAL LEAFSPOTS OF PENNSYLVANIA TOBACCO[*]

I. Occurrence and nature of the microorganism associated with wildfire

J. J. REID, J. NAGHSKI, M. A. FARRELL, and D. E. HALEY[†]

THE CONSTANT THREAT of serious loss from leafspot diseases has confronted Pennsylvania tobacco growers for a number of years, and continues to be regarded today as the major hazard of the industry.

Following serious outbreaks in 1917 it was found *(23 and 24)* that the more important of these diseases, "wildfire", was associated with the activities of *Bacterium tabacum.* A second leafspot disease of tobacco was reported *(7 and 8)* which was named "angular leafspot" or "blackfire", in the same year. The organism associated with this disease was called *Bacterium angulatum.* The influence of climate, plant nutrition, and agronomic practices on the severity of these leafspot diseases was noted by these early investigators. Both groups of workers *(8 and 23)*, however, concluded that these diseases had their origin in the seedbed and were carried to the field on the transplants.

For this reason all attempts at controlling the leafspot diseases of tobacco in the past two decades have been primarily attempts to eliminate from the seedbed the bacteria associated with the disease. The practices recommended have been termed "measures of sanitation" and have included removal of the seedbed from the vicinity of buildings, the steaming of soil, the disinfection of seed and implements, and the use of fungicides such as bordeaux mixture on both soil and plant. In certain districts producing a low nitrogen leaf tobacco, recommendations *(5, 18 and 19)* with respect to the nutrition of the plant have contributed to the control of disease.

The leafspot problem in the Lancaster area today continues to be serious. Sanitation apparently has failed to fully control the trouble, and the nutritional recommendations applicable in certain other districts are thought to be of little or no value in the production of cigar leaf and other high nitrogen tobaccos. This bulletin deals with the nature and identity of the microorganisms associated with leafspot diseases of tobacco, the influence of soil steaming on these microorganisms, and the normal bacterial flora of tobacco and other plants.

---

[*] Authorized for publication February 11, 1942.

[†] Associate Professor of Bacteriology, Graduate Student of Bacteriology, Professor and Head of Bacteriology, and Professor of Soil and Phytochemistry, respectively.

BACTERIOLOGICAL METHODS

1. Analysis of soil samples.—Bacteriological examination of soil samples was made in the following manner: Soils were plated on sodium caseinate (nutrose) agar to determine total bacterial numbers. Fungi were determined by means of plate counts on glucose-peptone agar adjusted to pH 4.5. The numbers of green fluorescent bacteria were determined by the inoculation of asparagin-mineral salts broth to which crystal violet had been added to inhibit the growth of spore formers. The broth was inoculated with soil dilutions of 1 to 10, 1 to 50, 1 to 100, and 1 to 200, and the highest dilution in which growth of green fluorescent organisms occurred was used in calculating the minimum number of such organisms present in the sample. All incubations were carried out at 28° Centigrade.

The agglutination test was employed in the examination of green fluorescent bacteria for the presence of wildfire and angular leafspot organisms. Asparagin-mineral salts-crystal violet broth which showed growth of green fluorescent organisms was streaked on agar plates, colonies were picked and transferred to agar slants, and by means of the standard agglutination technique these subcultures were checked for evidence of agglutination in the presence of specific immune sera. Immune sera were prepared by the immunization of rabbits with known strains of the wildfire and angular leafspot organisms which had been obtained from other investigators. Agglutination titres of less than 1 to 300 were not considered specific. At frequent intervals strains identified by means of the agglutination technique were tested on a susceptible host as a further check on the identity of the organism.

2. Analyses of plant material.—Plant material was plated on nutrient agar plates for total numbers of bacteria, and from these plates green fluorescent colonies were picked and made the subject of special study. In those instances in which colonies of green-fluorescent organisms were not observed on nutrient agar the use of asparagin-mineral salts broth was resorted to. This substrate was used in the manner described in the discussion of soil analyses.

3. Cultures employed.—The bacterial cultures employed throughout this study were maintained on nutrient agar slants, pH 7.2, with frequent transfer and stored at 4° Centigrade.

4. Morphological and physiological characteristics.—The numerous morphological, cultural, and biochemical characteristics were determined in the usual manner with minor modifications which are noted in the thesis of one of the authors *(21)*.

5. Single-cell isolations.—Most of the cultures used as antigens in this study were the progeny of single cells obtained by the use of the Chambers micromanipulator. The Chambers technique as modified by Wright and McCoy *(25)* and Wright and Nakajima *(26)* with a few minor changes was used in this study. Cultures in the logarithmic growth phase (4 to 8 hours old) were taken up with a sterile micropipette and a series of droplets made on the underside of a sterile coverslip suspended above a moist chamber. The droplets were immediately examined microscopically and all droplets containing one cell were carefully charted. Incubation was carried out on the coverslip above the moist chamber and transfers were made to larger quantities of substrate after reproduction of the cells had occurred.

6. Serological techniques.—

A. PREPARATION OF ANTISERA:

Antigens for animal immunization were prepared by growing cultures in flasks with large agar surfaces. The flasks were inoculated with one-half ml of 18-hour broth cultures and incubated at room temperature for a period of 18 to 24 hours. The growth was checked for purity and then was washed off the agar with saline solution (0.85 per cent NaCl) containing 0.3 per cent tricresol (C.P.). The cells were removed from saline solution by centrifugation, were resuspended in saline solution, and again removed by centrifugation. Following this washing technique the cells were suspended in sufficient saline solution to obtain a density comparable to a No. 4 McFarland nephelometer standard, and permitted to remain for a period of 48 hours at 4° C.

Rabbits selected for immunization weighed not less than 2 kilograms each and were rejected in those cases in which the normal serum showed agglutinins at a dilution of 1 to 20. The animals received the antigens by intravenous injection. The initial dose was 0.25 ml The dosage was increased daily by 0.25 ml until the maximum dose of 2 ml was reached. The maximum dose was given daily until the tenth day. Following this series of ten injections of killed antigen, a series of injections of living antigen was administered. The initial dose of the living antigen was 0.25 ml. This was increased by 0.25 ml daily until a total of 6 ml of the living antigen had been administered. Following a 4-day rest period a trial bleeding was made. If a satisfactory titre had been reached, 30 to 40 ml of blood were removed aseptically from the heart, and allowed to clot; the serum was removed and stored under sterile conditons at 4°C.

B. AGGLUTINATION TECHNIQUE:

Sera dilutions from 1-20 to 1-10,240 were made in 1 ml portions of 0.85 per cent saline solution containing 0.3 per cent tricresol. One ml of the antigen suspension was added to each tube, giving final sera dilutions of from 1-40 to 1-20,480. The antigens were prepared from 18 to 24-hour cultures grown on nutrient agar which was removed and washed as described under the preparation of antisera and adjusted to a density comparable to a 1.5 to 2 McFarland nephelometer standard. Sensitization was carried out at 37°C. for a period of 24 hours followed by 12 hours at 4°C. and the degree of macroscopic agglutination recorded.

C. AGGLUTININ ABSORPTION:

The technique as outlined by Krumwiede, Cooper, and Provost *(16)* was followed. Large quantities of cells were grown at room temperature on nutrient agar for 25 hours, washed off with saline solution, filtered through cotton, and the cells recovered by centrifugation. The cells were again washed with saline solution and recovered by centrifugation. To a measured volume of the washed cells, serum and diluent were added according to the following formula:

$$(\text{MASS OF CELLS} - 10\ \text{PER CENT}) \times \text{DOSE FACTOR} = \text{TOTAL VOLUME}$$

$$\frac{(\text{MASS} - 10\ \text{PER CENT}) \times (\text{DOSE FACTOR} - 1)}{\text{SERUM DILUTION FACTOR}} = \text{SERUM REQUIRED}$$

$$\text{TOTAL VOLUME} - (\text{MASS OF CELLS} + \text{SERUM}) = \text{VOLUME OF DILUENT}$$

Trial absorption titrations were performed on each serum to determine optimal dose, serum dilution, and number of absorptions. A practice of using two absorbing doses was adopted as it resulted in a saving of cells and yielded more complete and uniform absorption. To avoid difficulty with differences in absorptive abilities of herterologous cultures, the absorbing dose was twice that necessary to completely absorb the homologous antiserum. As the absorptive capacity of cells in the "S" phase was found in this study to be less than that of cells in the "M" phase, three absorptions were made when absorbing with "S" cells.

The serum-bacteria suspensions were incubated at 37°C. for 3 hours with shaking every 30 minutes and were then kept overnight at 4°C. The cells were then removed by centrifugation, and to the supernatant serum another calculated volume of washed cells was added and the absorption again carried out. The cells were again removed by centrifugation, and in the case of absorption with "M" phase cells the serum was tested for degree of absorption by the standard macroscopic agglutination test previously described. In the case of absorption by "S" cells another calculated volume of washed cells was added and absorption carried out for the third time before making the agglutination test. As a control, identical serum dilutions to which no absorbing cells were added were carried under the same conditions and finally tested by the standard agglutination technique.

7. Production of Dawson "S" forms.—The cultures in the "M" phase were grown in nutrient broth containing 10 per cent of the homologous "M" antiserum. Transfers were made daily and for the purpose of colony examination nutrient agar streak plates were also made daily from the broth containing the antiserum. In most cases examination of the streak plates showed that "S" forms appeared in abundance following the third or fourth serial transfer in immune serum broth. In the case of some cultures, however, prolonged serial transfer in the immune serum broth proved necessary.

Isolations were made from typical "S" colonies and antigens prepared for test against the homologous "M" phase antiserum. Failure to show agglutination at a titre of 1-320 was used as an arbitrary criterion of the removal of the capsule, at which time the culture was considered as representative of the "S" phase. Preparation of antisera in the case of the "S" phase antigens was carried out in the manner described in the case of "M" phase antigens.

EXPERIMENTAL RESULTS SHOWING NATURE OF ORGANISM

Morphological and physiological characteristics of green fluorescent isolates.—In the course of these studies of the microflora of soils and plants, isolations of more than 4000 strains of gram-negative fluorescent bacteria have been made in the bacteriological laboratories of The Pennsylvania State College. In addition approximately 100 cultures of *Pseudomonas* and *Phytomonas* were obtained from other laboratories and used for purposes of comparison. From this large number, 640 representative cultures were selected for study in this investigation. The morphological and biochemical characteristics of this group of bacteria were determined by employing the following tests: Staining for flagella, Gram reaction, hemolytic power, nitrate reduction, gelatin liquefaction, action in litmus milk, fermentation of glucose, of sucrose and of lactose, indol formation, hydrogen sulfide production, growth on potato, growth at different temperatures, and pigment formation.

These 640 green fluorescent bacteria were divided into two groups on the basis of the characteristics shown in table 1.

Table 1 shows that a small number of the 640 cultures were monotrichous, possessing only one polar flagellum, while 613 cul-

Table 1.—Classification of 640 fluorescent bacteria studied.

	MONO-TRICHOUS	LOPHO-TRICHOUS
Number of cultures	27	613
Per cent showing hemolytic activity	100.0	0.0
Per cent producing pyocyanin	63.0	0.0
Per cent showing reduction of nitrates	100	100.0
Per cent exhibiting growth at 37°C.	100	1.6
Per cent showing gelation liquefaction	100	98.4

tures showed 2 to 5 polar flagella or were lophotrichous. The type of flagellation was found to be unchanged by cultivation on the various media used in this study, and, although it was possible to effect the temporary removal of flagella by growing in broth containing phenol in appropriate concentrations, transfers from such broth resulted in a return to the original type of flagellation. All monotrichous strains were found to be actively hemolytic while none of the 613 lophotrichous forms were capable of lysing bovine or sheep red blood cells. Pyocyanin formation was shown only with certain of the 27 montrichous forms and only a small percentage of the lophotrichous types which did not liquefy gelatin were able to grow at 37°C. Nitrate reduction contrary to the findings of some workers *(9 and 24)* was found to occur with all of the 640 cultures.

On the basis of these as well as additional morphological and cultural characteristics, it was evident that the 27 monotrichous cultures conform to the description of *Bacterium pyocyaneum* Lehmann and Neumann *(17)*. This group of 27 strains also conforms to the description of *Pseudomonas aeruginosa* as given by Bergey et al. *(1)* with the exception of flagellation. Inasmuch as a number of the strains in this group were received from other laboratories as *Pseudomonas aeruginosa* and had originally been isolated from human lesions, the entire group of 27 has been considered representative of that species. Subsequently, Harris *(12)* found all members of this group to be serologically homologous in the decapsulated state and antigenically different from decapsulated representatives of the 613 lophotrichous strains.

Additional morphological and biochemical characteristics of the 613 lophotrichous forms permits their separation into 2 groups as shown in table 2.

Table 2.—The morphological and biochemical characteristics of 613 lophotrichous bacteria.

	GROUP 1	GROUP 2
Number of cultures	603	10
Polar flagella	2 to 5	2 to 5
Gram reaction	Negative	Negative
Shape	Short rods	Short rods
Growth at 37°C.	Little or no growth	Good growth
Agar slant	Filiform, greyish white	Filiform, greyish white
Gelatin stab	Crateriform to infundibuliform liquefaction	No liquefaction
Nutrient broth	Turbid, slimy sediment and pellicle	Turbid, slimy sediment and pellicle
Litmus milk	Alkaline with clearing	No change
Pigment production:		
Pyocyanin	—	—
Fluorescein	+	+
Fermentation:		
Glucose	+	+
Sucrose	+ (few —)	+ (few —)
Lactose	—	—
Reduction of Nitrates	+	+
Indol	—	—
H_2S	—	—

— = Negative: + = Positive.

It may be noted in table 2 that the 613 lophotrichous organisms were separated into 2 groups on the basis of their action in gelatin, litmus milk, and the ability to grow at 37°C. Group 1 comprising 603 cultures was characterized by either very feeble growth or no growth at 37°C., rather active liquefaction of gelatin, and peptonization of litmus milk. Group 2 containing 10 strains was found to grow well at 37°C. but did not liquefy gelatin or bring about any change in litmus milk. All lophotrichous forms were found to grow well at room temperature on nutrient agar slants. Growth was found to be filiform, abundant, and greyish-white. After an incubation period of a few days, a soluble yellow-green pigment penetrated the substrate, increasing in amount as aging progressed. In nutrient broth growth was initiated at the surface as delicate floccules which as they settled resulted in uniform turbidity. After incubation for several days a slightly viscid sediment accumulated. A yellow-green pigment was usually in evidence near the surface of the broth. As this pigment was colorless under reduced conditions, it was not apparent throughout the entire tube as long as the culture was actively metabolizing. On aging of the cultures, the pigment was observed to travel downward as oxygen apparently diffused downward and oxidized the pigment to a colored state. The use of synthetic media showed that all lophotrichous strains produced fluorescein, but in no case could production of pyocyanin be demonstrated. Careful control of pH was found necessary in this study as fluorescein decolorizes in slightly acid solution and may be overlooked under such conditions.

Group 1 forms were found to liquefy gelatin with an initial crateriform type of liquefaction that slowly changed to the infundibuliform type. In most cases liquefaction was found to be pronounced at the end of three days at 18°C. Group 2 cultures showed no evidence of liquefaction in any trial at the end of 14 days.

Activity in litmus milk was found closely correlated with activity in gelatin. Group 1 was found to produce an alkaline reaction followed by clearing. In undisturbed tubes the alkaline reaction first appeared as a zone at the top and progressed downward. As the intensity of the daily zones was distinctly pronounced the medium took on a banded appearance. Clearing also began at the top of the tube and progressed downward. Reduction of litmus began at the bottom and after the fifth to seventh day was usually complete except for a shallow zone at the top of the tube. At the end of a week the milk usually became completely cleared and assumed a greenish-yellow color. Group 2 on the other hand did not produce a noticeable change in litmus milk.

The production of acid from glucose was observed in both groups of lophotrichous cultures. In nutrient broth, to which glucose was added, the medium turned slightly alkaline within one day but as incubation proceeded the medium slowly became acid, maximum acidity being reached in from three to five days. Following this the medium again became alkaline, and at the tenth day all signs of previous acidity had been obscured. The maximal acidity in glucose nutrient broth, although evident, was very slight, and was obscured if the original reaction of the substrate was slightly alkaline. Certain lophotrichous forms appeared to be able to attack sucrose. The acid from sucrose was found to appear more slowly than in the case of glucose but the acidity remained over a longer period of time. Lactose was not attacked by the members of either group.

All strains were found to be active denitrifiers. A few lophotrichous strains were found able to reduce the nitrogen of nitrates to the elemental form. Production of indol and hydrogen sulfide

were never observed with any of the 613 cultures. On potato slants all cultures grew luxuriently, producing a dirty green coloration.

These data indicate that the group of 603 cultures conformed to the description of *Bacterium fluorescens* (Flugge), Lehmann and Neumann *(17)*, although it includes isolations received from other laboratories as *Phytomonas tabaci, Ph. angulata, Ph. primulae, Ph. cerasi, Ph. vignae, and Ph. syringae,* as well as isolations received as *Pseudomonas fluorescens.* The other group of 10 cultures conformed to the description of *Bacterium putidum* (Flugge), Lehman and Neumann *(17)* and consisted entirely of organisms isolated from soil during the course of this investigation.

Serological studies.—With the discovery of the agglutination phenomena by Gruber and Durham *(10)* in 1896, a new means has been available for the identification of bacteria. Following this discovery, serological reactions on account of their great sensitivity and specificity have been employed to a large extent in assisting in disease diagnosis as well as in the species identification of bacteria. The union of bacterial cells with their respective antibodies (agglutinins) brings about a disappearance of the agglutinins from the serum in which they were present. Such a test known as agglutinin absorption as employed in this study, was first used clinically by Castellani *(3)* in 1902 and was established by Krumweide, Cooper, and Provost *(16)*. These authors state:

> "We have emphasized again and again the postulate that a completely positive reciprocal agglutinin absorption test is the ultimate criterion of serological likeness or identity of two bacterial cultures or strains and that absence of the completely positive reciprocal reaction is a definite criterion of unlikeness."

Although 603 of the 613 lophotrichous organisms were found to be similar to *Pseudomonas fluorescens* on the basis of morphology and biochemical reactions, this group included cultures previously identified as other species in the genus *Pseudomonas* as well as species in the genus *Phytomonas.* In a further effort to determine whether these 603 cultures formed a homogeneous group, serological reactions such as agglutination and agglutinin absorption were employed.

For the purpose of these serological studies 25 representative cultures including 6 recognized *Phytomonas* species, 8 cultures of *Pseudomonas fluorescens* and 1 culture of *Pseudomonas putida* were used. The source of these cultures is shown in table 3.

Table 3.—Source of cultures used in serological studies.

CULTURE NO.	SOURCE	ISOLATED BY
BB	*Ph. tabaci* isolated from wildfire lesion, 1938	W. S. Beach, The Pennsylvania State College
11	*Ph. tabaci* isolated from wildfire lesion, 9/1937	W. S. Beach, The Pennsylvania State College
12	*Ph. tabaci* isolated from wildfire lesion, 2/1938	W. S. Beach, The Pennsylvania State College
527	*Ph. tabaci* isolated from wildfire lesion	E. E. Clayton, U. S. Department of Agriculture
16	*Ph. tabaci* isolated from wildfire lesion, 1923.	American Type Culture Collection
410	*Ph. angulata* isolated from angular leafspot, 1939	J. Johnson, University of Wisconsin
519	*Ph. primulae* isolated from leafspot of primrose	P. A. Ark, University of California
520	*Ph. cerasi* obtained from E. E. Wilson, University of California	W. H. Burkholder, Cornell University
524	*Ph. syringae* isolated from lima beans, 10/1937	W. H. Burkholder, Cornell University
204	*Ps. fluorescens* isolated from normal mature red clover	J. Naghski, The Pennsylvania State College
80	*Ps. fluorescens* isolated from normal tobacco, 1938	R. G. Harris, The Pennsylvania State College
521	*Ph. vignae* obtained from E. E. Wilson, University of California	W. H. Burkholder, Cornell University
353	*Ps. fluorescens* isolated from soil	J. Naghski, The Pennsylvania State College
504	*Ps. fluorescens* isolated from old red clover, 1939	J. Naghski, The Pennsylvania State College
630-1	*Ps. putida* isolated from compost heap, 1939	J. Naghski, The Pennsylvania State College
BBF	Single cell of *Ph. tabaci* BB isolated from wildfire	W. S. Beach, The Pennsylvania State College
80F$_2$	Single cell of *Ps. fluorescens* 80 isolated from normal tobacco, 1938	R. G. Harris, The Pennsylvania State College
204D	Single cell of *Ps. fluorescens* 204 isolated from normal mature red clover	J. Naghski, The Pennsylvania State College
519F	Single cell of *Ph. primulae* 519 isolated from leafspot of primrose	P. A. Ark, University of California
520A	Single cell of *Ph. cerasi* 510 obtained from E. E. Wilson, University of California.	W. H. Burkholder, Cornell University
521L	Single cell of *Ph. vignae* obtained from E. E. Wilson	W. H. Burkholder, Cornell University
524C	Single cell of *Ph. syringae* 524 isolated from lima beans, 10/1937	W. H. Burkholder, Cornell University
230	*Ps. fluorescens* isolated from normal young red clover, 1939	J. Naghski, The Pennsylvania State College
248	*Ps. fluorescens* isolated from normal young red clover, 1939	J. Naghski, The Pennsylvania State College
353	*Ps. fluorescens* isolated from soil	J. Naghski, The Pennsylvania State College

In a preliminary series of agglutination experiments two cultures in the Dawson "M" phase were used as antigens in animal inoculation for preparing agglutinating sera. These cultures were *Phytomonas tabaci* BB and *Pseudomonas fluorescens* 204. The agglutination reactions using the antisera so obtained with homologous and heterologous cultures are shown in table 4.

Table 4.—Agglutination reactions of "M" phase Phytomonas and Pseudomonas species with anti-sera prepared against a strain of Ph. tabaci and a strain of Ps. fluorescens in the "M" phase.

	PHYTOMONAS TABACI BB ANTI-SERUM DILUTIONS									PSEUDOMONAS FLUORESCENS 204 ANTI-SERUM DILUTIONS								
SPECIES OF BACTERIA	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800*	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800*
Ph. tabaci BB	4†	4	4	4	4	4	3	2	–	–	2	4	4	4	3	1	–	–
Ph. tabaci 11	4	4	4	2	–	–	–	–	–	2	4	2	1	–	–	–	–	–
Ph. tabaci 12	4	4	4	4	4	3	1	–	–	1	2	4	4	2	–	–	–	–
Ph. tabaci 527	4	4	4	4	4	4	4	2	–	–	2	4	4	4	3	2	–	–
Ph. tabaci 16	4	4	4	2			–	–	–	–	2	2	–	–	–	–	–	–
Ph. angulata 410	4	4	4	4	4	4	4	2	–	–	2	4	4	4	3	1	–	–
Ph. primulae 519	4	4	4	4	4	4	2	1	–	–	2	4	4	4	2	–	–	–
Ph. cerasi 520	4	4	4	4	4	4	4	2	–	2	4	4	4	4	4	2	–	–
Ph. vignae 521	4	4	4	2	–	–	–	–	–	2	4	4	4	2	–	–	–	–
Ph. syringae 524	4	4	4	4	2	–	–	–	–	2	4	4	4	3	1	–	–	–
Ps. fluorescens 204	–	4	4	4	4	4	2	1	–	4	4	4	4	4	4	4	2	–
Ps fluorescens 80	4	4	4	4	4	3	1	–	–	2	4	4	4	4	4	2	–	–
Ps. fluorescens 353	4	2	–	–	–	–	–	–	–	4	2	–	–	–	–	–	–	–
Ps. fluorescens 504	4	4	4	4	4	4	4	2	–	4	4	4	4	4	4	4	3	1
Ps putida 630-1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

* Higher anti-serum dilutions and saline control were negative.

† Numbers refer to degree of macroscopic agglutination: 4 = complete; 1 = slight; – = no agglutination.

It will be noted in table 4 that all members of lophotrichous group 1 used in this experiment showed some antigenic relationship with each of the antisera, while *Ps. putida* 630-1 representing group 2 of the lophotrichous organism failed to show such antigenic relationship. It will also be noted that various strains of *Phytomonas tabaci* reacted in much lower dilution with the antiserum prepared with *Pseudomonas fluorescens* 204 than did *Phytomonas tabaci* BB. In this connection it is noteworthy that the two strains of *Phytomonas tabaci* showing the lowest titre against both antisera, strains 11 and 16 are the oldest isolations in the group and have been in laboratory cultivation for a much longer period of time than have any of the others. Strain 12 represents an isolation made more recently than 11 and 16 but not as recently as BB and strain number 527. The relationship between length of laboratory cultivation and degree of reaction with the two antisera is also apparent with other species of *Phytomonas*. *Ph. vignae* 521 and *Ph. syringae* 524 are relatively old isolations and reacted poorly while, on the other hand, the other species of *Phytomonas* represented are more recent isolations and reacted to the titer of BB and 204. Antigenic differences in the case of *Pseudomonas fluorescens* strains, however, cannot be explained on this basis as all are relatively recent isolations.

In order to confirm the similarity in antigenic nature noted between *Phytomonas tabaci* BB and *Pseudomonas fluorescens* 204 as shown in table 4, an agglutinin absorption study was made using these organisms and their homologous antisera. The data obtained are presented in table 5.

Table 5.—Reciprocal agglutinin-absorption of "M" phase anti-sera and "M" phase antigens of Phytomonas tabaci BB and Pseudomonas fluorescens 204.

SERUM	ABSORBED BY	AGGLUTINATED WITH	SERUM DILUTIONS 1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	C
BB	BB	BB	-	-	-	-	-	-	-	-	-
		204	-	-	-	-	-	-	-	-	-
BB	204	BB	-	-	-	-	-	-	-	-	-
		204	-	-	-	-	-	-	-	-	-
204	BB	BB	-	-	-	-	-	-	-	-	-
		204	-	-	-	-	-	-	-	-	-
204	204	BB	-	-	-	-	-	-	-	-	-
		204	-	-	-	-	-	-	-	-	-

C = saline control.

The inability to demonstrate, table 5, either homologous or heterologous agglutination between *Phytomonas tabaci* BB and *Pseudomonas fluorescens* 204 after reciprocal absorption in titres as low as 1 to 50 when it was previously shown, table 4, that cross agglutination titres ranged from 1-800 to 1-3,200 indicates the very close antigenic relationship and similarity of these two microorganisms.

Serological studies employing single-celled cultures.—In order to make certain that the antigenic relationship found to exist among known strains of *Pseudomonas* and *Phytomonas* in these preliminary studies was not a result of impure cultures, 39 single celled isolations were made of 5 species of *Phytomonas* and 3 species of *Pseudomonas* by the method previously described. Only those single-cell strains were chosen for use as antigens which closely resembled the parent culture in all morphological, physiological, and serological characteristics, table 3. Representative single-cell strains in the Dawson "M" phase were used as antigens in animal inoculation and the agglutination reactions of these strains with the antisera obtained are presented in table 6.

Table 6.—Cross agglutination reactions of the "M" phase of Pseudomonas and Phytomonas species.

ANTIGENS	ANTISERA BBF	$80F_2$	410	204D	519F	520A	521L	524C	412
BBF	2560†	5120	2560	2560	2560	5120	—	—	—
$80F_2$	2560	5120	2560	2560	5120	5120	—	—	—
410	2560	5120	2560	2560	5120	10240	—	—	—
204D	1280	5120	1280	2560	5120	5120	—	—	—
519F	1280	5120	1280	2560	5120	10240	—	—	—
520A	1280	5120	2560	2560	5120	10240	—	—	—
521L	320	640	160	1280	160	2560	10240	2560	—
524C	640	1280	160	80	80	40	5120	10240	—
412*	—	—	—	—	—	—	—	—	1280

* 412 is a culture of *Ps. aeruginosa* used for purposes of comparison.
† Numbers represent the reciprocal of the highest serum dilution showing macroscopic agglutination.

The data in table 6 shows that the strains of *Phytomonas tabaci* BBF, *Ph. angulata* 410, *Ph. primulae* 519F, *Ph. cerasi* 520A and *Pseudomonas fluorescens* 204D and $80F_2$ give cross agglutination reactions in almost the tame titre as obtained with the homologous organism. *Phytomonas vignae* 521L and *Ph. syringae* 524C, on the other hand, show considerably less antigenic similarity.

Agglutinin absorption tests employing the single-celled isolations were also used in a further effort to determine relationships within this group of cultures. The data obtained are presented in table 7.

Table 7.—Reciprocal agglutinin absorption of "M" anti-sera by "M" antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	$80F_2$	410	204D	519F	520A	521L	524C
BBF	BBF	— 40*	— 40	— 40	+ 40	— 40	+ 40	— 40	— 40
	$80F_2$	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	410	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	204D	+ 640	+ 640	+ 160	— 40	— 40	— 40	— 40	— 40
	519F	+ 320	+ 320	— 40	— 40	— 40	— 40	— 40	— 40
	520A	+ 640	+ 640	+ 160	+ 40	— 40	— 40	— 40	— 40
	521L	+ 1280	+ 1280	+ 1280	+ 640	++ 640	++ 320	— 40	— 40
	524C	+ 1280	++ 1280	+ 1280	+ 640	+ 640	++ 160	— 40	— 40

* Numbers in this table are reciprocals of highest serum dilution showing any macroscopical agglutination. ++++, +++, ++, + denote degree of agglutination from complete to trace. Controls and higher dilutions were negative.

Table 7. (Continued)—Reciprocal agglutinin absorption of "M" anti-sera by "M" antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	80F₂	410	204D	519F	520A	521L	524C
80F₂	BBF	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	80F₂	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	410	— 40	— 40	— 40	++ 40	— 40	++ 40	— 40	— 40
	204D	+ 80	++ 320	+ 80	— 40	— 40	— 40	— 40	— 40
	519F	+ 640	++ 320	++ 160	— 40	— 40	— 40	— 40	— 40
	520A	+ 640	++ 320	+ 320	+ 40	— 40	— 40	++ 320	+ 160
	521L	++ 2560	+ 5120	++ 5120	++ 2560	+ 5120	+ 2560	— 40	— 40
	524C	+ 2560	++ 2560	+ 5120	+ 2560	+ 5120	++ 5120	— 40	— 40
410	BBF	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	80F₂	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	410	— 40	— 40	— 40	++ 40	— 40	++ 40	— 40	— 40
	204D	++ 320	+ 320	+ 160	— 40	— 40	— 40	— 40	— 40
	519F	++ 320	+ 160	— 40	— 40	— 40	— 40	— 40	— 40
	520A	+ 640	+ 640	+ 160	+ 40	— 40	— 40	— 40	— 40
	521L	+ 1280	++ 1280	++ 640	++ 640	+++ 640	— 40	— 40	— 40
	524C	++ 1280	+ 1280	+ 1280	+ 640	+++ 320	+ 1280	— 40	— 40
204D	BBF	+ 40	++ 40	+ 80	++ 160	++ 80	+ 160	+ 320	— 40
	80F₂	— 40	— 40	— 40	+ 160	— 40	+ 160	+ 320	— 40
	410	— 40	— 40	— 40	+ 640	— 40	+ 160	+ 320	— 40
	204D	+ 40	+ 40	— 40	— 40	— 40	— 40	+ 40	— 40
	519F	+ 80	— 40	— 40	+ 320	— 40	++ 80	++ 320	— 40
	520A	+ 160	++ 160	++ 40	++ 320	+ 40	— 40	+++ 320	+ 40
	521L	++ 1280	+++ 1280	+++ 1280	++ 1280	++ 1280	+ 1280	— 40	— 40
	524C	+ 1280	+ 1280	+++ 1280	+ 1280	+ 1280	+ 1280	— 40	— 40

Table 7. (Continued)—Reciprocal agglutinin absorption of "M" anti-sera by "M" antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	80F₂	410	204D	519F	520A	521L	524C
519F	BBF	+ 40	— 40	+ 40	+ 160	++ 80	+ 40	— 40	— 40
	80F₂	— 40	— 40	— 40	+++ 640	+++ 640	— 40	— 40	— 40
	410	40	40	— 40	++ 320	+++ 640	— 40	— 40	— 40
	204D	— 40	— 40	— 40	— 40	++ 320	— 40	— 40	— 40
	519F	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	520A	— 40	+ 40	+ 40	+ 160	++ 160	— 40	— 40	— 40
	521L	++ 1280	+ 2560	++ 2560	+ 2560	+ 2560	+ 2560	— 40	— 40
	524C	+++ 1280	+++ 1280	++ 2560	++ 1280	++ 1280	++ 1280	— 40	— 40
520A	BBF	— 40	++ 80	++ 80	++ 320	+ 160	++ 320	+ 80	— 40
	80F₂	— 40	— 40	++ 80	+ 160	+ 80	++ 160	+ 320	— 40
	410	— 40	— 40	— 40	+ 40	— 40	++ 40	++ 80	— 40
	204D	— 40	— 40	— 40	— 40	— 40	+ 160	++ 160	— 40
	519F	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	520A	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	521L	+ 5120	+ 5120	+ 10240	+ 10240	+ 5120	++ 2560	— 40	— 40
	524C	++ 2560	+ 5120	+ 5120	+ 5120	++ 1280	++ 5120	+ 640	— 40
521L	BBF	— 40	— 40	— 40	+ 160	— 40	+ 80	+ 1280	++ 320
	80F₂	— 40	— 40	— 40	— 40	— 40	— 40	+++ 2560	+ 2560
	410	— 40	— 40	— 40	— 40	— 40	— 40	++ 2560	+ 1280
	204D	— 40	— 40	— 40	— 40	— 40	— 40	++ 5120	++ 5120
	519F	— 40	— 40	— 40	— 40	— 40	— 40	+ 2560	+ 2560
	520A	— 40	— 40	— 40	+ 320	+++ 40	— 40	+++ 1280	+ 1280
	521L	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	524C	— 40	— 40	— 40	+ 160	— 40	++ 40	++ 1280	— 40

Table 7. (Continued)—Reciprocal agglutinin absorption of "M" anti-sera by "M" antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	 80F₂	 410	 204D	 519F	 520A	 521L	 524C
524C	BBF	— 40	— 40	— 40	— 40	— 40	+ 40	+ 640	+ 640
	80F₂	— 40	— 40	— 40	— 40	— 40	— 40	+ 640	+ 2560
	410	— 40	— 40	— 40	— 40	— 40	— 40	++ 640	+ 2560
	204D	— 40	— 40	— 40	— 40	— 40	— 40	— 40	++ 5120
	519F	— 40	— 40	— 40	— 40	— 40	— 40	+ 640	+ 2560
	520A	— 40	— 40	— 40	— 40	++ 80	— 40	++ 640	+ 2560
	521L	— 40	— 40	— 40	— 40	— 40	— 40	— 40	++ 640
	524C	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40

It will be noted in table 7 that reciprocal absorption (employing these cultures) indicates the antigenic identity of *Ph. tabaci* BBF, *Ph. angulata* 410, *Ph. primulae* 519F, *Ph. cerasi* 520A and *Pseudomonas fluorescens* 80F₂ and 204D. *Ph. vignae* 521L and *Ph. syringae* 524C were found to reduce the titres of the other sera although not effecting complete removal of the agglutinins. The other cultures likewise did not completely remove the agglutinins from the *Ph. vignae* 521L and *Ph. syringae* 524C antisera, although effecting a reduction in titre. On the basis of these data, *Phytomonas syringae* 524C and *Ph. vignae* 521L appear to have a greater antigenic similarity to each other than either exhibits toward the remaining cultures. Thus in the "M" phase, they cannot be considered identical. It should be mentioned again, however, that the strains of *Phytomonas vignae* 521L and *Ph. syringae* 524C are the only cultures in this comparison which are not recent isolations. Previous work with *Phytomonas tabaci* strains which had been under laboratory cultivation for long periods of time indicated a progressive loss of antigenic similarity in the "M" phase as a result of laboratory cultivation, table 4. The data presented in tables 4 to 7 inclusive obtained from the study of organisms in the Dawson "M" phase indicates the very close antigenic relation of these cultures.

Antigenic relation of decapsulated green fluorescent bacteria.—The studies of Julianelle *(14)* have shown that antigenic relationships between cultures in the "M" phase may merely indicate that similar soluble specific polysaccharides are present in the capsular material of the cells and that upon decapsulation the same cells show no antigenic similarity. For the purpose of determining whether the antigenic similarity found in this study was merely one of capsular material or actually involved the proteins of the cell, Dawson "S" forms of these strains of *Phytomonas* and *Pseudomonas* were obtained by growth of the organism in nutrient broth containing 10 per cent homologous immune serum. In the case of recent isolations included in this study little difficulty was encountered in obtaining the Dawson "S" phase. In the case, however, of the older isolates, *Phytomonas vignae* 521L and *Phytomonas syringae* 524C, considerable difficulty was encountered not only in obtaining the "S" phase but also in maintaining organisms in it. In these two cases the difficulty in maintaining the cells in the "S" phase was so great that the cultures finally used as antigens cannot be said with certainty to represent the "S" phase, as "M" variants appeared frequently in spite of all precautions.

Animals were immunized with the "S" phase strains and the antisera obtained were used in making agglutination tests with homologous and heterologous strains. The results are presented in table 8.

Table 8.—Cross agglutination reaction of the "S" phase of Pseudomonas and Phytomonas species.

ANTIGENS	ANTISERA BBF	 80F₂	 410	 204D	 519F	 520A	 521L	 524C	 412
BBF	10240†	10240	10240	5120	5120	10240	5120	5120	—
80F₂	5120	10240	10240	5120	5120	10240	5120	5120	—
410	5120	10240	10240	5120	5120	5120	5120	5120	—
204D	5120	5120	10240	5120	10240	5120	5120	5120	—
519F	5120	10240	5120	5120	10240	10240	2560	5120	—
520A	5120	10240	10240	5120	10240	10240	5120	5120	—
521L	5120	5120	5120	10240	10240	10240	10240	5120	—
524C	5120	5120	10240	10240	5120	5120	5120	10240	—
412*	—	—	—	—	—	—	—	—	1240

* 412 is a culture of *Pseudomonas aeruginosa* used for the purpose of comparison.

† Numbers represent the reciprocal of the highest serum dilution showing macroscopic agglutination.

It will be noted in table 8 that this group of lophotrichous green fluorescent bacteria in the "S" phase showed definite antigenic similarity upon cross agglutination among all strains while culture No. 412 a monotrichic culture which was used as a control showed no antigenic relation with the eight lophotrichous "S" phase antisera. The relationship between *Phytomonas syringae* 524C and *Ph. vignae* 521L and the remaining members of the lophotrichous group appeared more pronounced than in any study conducted with "M" phase cells.

The first series of agglutinin absorption reactions made with organisms in the "S" phase and the antisera obtained from animal inoculation with "S" phase cells indicated that the absorbing capacity of "S" phase cells was less than those in the "M" phase. The titres obtained upon homologous and heterologous agglutination showed that a similar degree of absorption had occurred but seldom had the agglutinins been completely removed. For this reason three standard absorbing doses of antigen were used in later studies. Eight antisera produced with "S" phase cultures were each absorbed by the eight cultures in the "S" phase and then tested for the presence of agglutinins with homologous and heterologous cultures. The results are shown in table 9.

Table 9.—Reciprocal agglutinin absorption of "S" phase anti-serum and "S" phase antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	80F₂	410	204D	519F	520A	521L	524C
BBF	BBF	++ 20*	+ 40	++ 20	+ 40	+ 80	++ 40	— 50	— 50
	80F₂	++ 80	+ 50	++ 20	+ 50	++ 50	+ 50	— 50	— 50
	410	++ 20	+ 50	++ 20	+ 40	++ 50	++ 40	+ 50	— 50
	204D	+ 40	— 50	++ 20	+ 20	— 50	++ 20	— 50	— 50
	519F	+ 50	++ 50	+ 40	+ 50	++ 20	+ 50	— 50	— 50
	520A	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50
	521L	++ 100	+ 100	+++ 50	+++ 50	+ 100	++ 100	++ 100	+ 100
	524C	+ 1600	+++ 1600	++ 1600	++ 1600	++ 1600	+++ 1600	— 50	— 50
80F₂	BBF	++ 40	+ 80	++ 40	+ 80	+ 40	+ 80	+ 50	++ 50
	80F₂	++ 50	++ 50	+ 50	— 50	++ 40	++ 50	+ 50	+ 50
	410	+ 80	++ 40	+ 40	— 50	— 50	++ 50	++ 50	++ 50
	204D	+ 40	++ 20	+ 80	+ 40	+ 40	++ 50	++ 50	+ 50
	519F	++ 50	+ 50	+ 50	+ 50	+ 50	— 50	— 50	++ 50
	520A	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50
	521L	+ 100	+ 50	— 50	— 50	++ 50	++ 50	+ 50	+ 50
	524C	++ 1600	+++ 1600	+++ 1600	+++ 1600	++ 3200	++ 3200	— 50	— 50

* Numbers are reciprocals of the highest serum dilution showing macroscopic agglutination. ++++, +++, ++, + denote degree of agglutination from complete to trace. Higher dilutions and saline controls were negative.

Table 9. (Continued)—Reciprocal agglutinin absorption of "S" phase anti-serum and "S" phase antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	80F₂	410	204D	519F	520A	521L	524C
410	BBF	+ 20	+ 40	+ 80	++ 20	++ 40	+ 40	— 50	— 50
	80F₂	++ 20	+ 20	++ 40	+ 40	+ 40	++ 20	— 50	— 50
	410	++ 20	++ 40	+ 80	+ 20	+ 80	+ 40	+ 50	+ 50
	204D	+ 20	+ 20	+ 20	+ 20	+ 40	++ 20	— 50	— 50
	519F	++ 50	++ 50	+ 50	— 50	— 50	++ 50	++ 50	+ 50
	520A	++ 50	++ 50	+ 100	++ 50	+ 50	+ 50	+ 100	+ 50
	521L	+ 100	+ 100	++ 50	+++ 50	+ 50	+ 100	+++ 50	++ 50
	524C	++ 3200	++ 3200	+++ 3200	++ 3200	+++ 3200	+++ 3200	— 50	— 50
204D	BBF	++ 40	+ 80	++ 40	+ 80	++ 40	+ 80	++ 50	++ 50
	80F₂	+ 20	+ 20	+ 20	+ 20	+ 50	+ 50	+ 50	+ 50
	410	+ 40	++ 40	++ 40	+ 40	+++ 50	++ 50	++ 50	++ 50
	204D	+ 20	+ 20	+ 20	+ 20	+ 50	+ 50	+ 50	++ 50
	519F	+++ 50	++ 50	+ 100	+ 100	++ 50	++ 50	++ 50	++ 50
	520A	+ 100	+++ 50	+++ 50	+ 100	+ 100	+++ 50	++ 50	++ 50
	521L	+++ 50	++ 50	+ 50	++ 50	+ 50	++ 50	++ 50	+ 50
	524C	+++ 1600	++ 800	++ 800	+++ 1600	++ 1600	++ 800	++ 50	— 50
519F	BBF	— 40	— 40	— 40	+ 80	+ 80	— 40	— 40	— 40
	80F₂	+++ 50	+ 50	+ 50	+ 50	+ 100	++ 50	++ 50	++ 50
	410	+ 80	++ 50	+ 50	+ 40	++ 50	++ 50	+ 50	+ 50
	204D	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	519F	+ 50	+ 50	++ 50	++ 50	++ 50	+ 50	— 50	+ 50
	520A	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	521L	+ 100	++ 50	++ 50	+ 100	+ 100	+ 100	+ 100	++ 50
	524C	++ 1600	+++ 800	+++ 800	++ 1600	++ 1600	++ 1600	— 50	— 50

Table 9. (Continued)—Reciprocal agglutinin absorption of "S" phase antiserum and "S" phase antigens of Pseudomonas and Phytomonas species.

SERUM	ABSORBED BY	ANTIGENS BBF	$80F_2$	410	204D	519F	520A	521L	524C
520A	BBF	+ 80	— 40	++ 40	+ 80	— 40	++ 80	+ 40	+ 40
	$80F_2$	— 40	— 40	— 40	+ 80	— 40	+ 80	— 40	— 40
	410	++ 40	+ 40	— 40	+ 80	+ 40	+ 80	— 40	— 40
	204D	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	519F	++ 100	+ 100	+ 100	++ 50	++ 50	+ 100	++ 50	++ 50
	520A	— 40	— 40	— 40	— 40	— 40	— 40	— 40	— 40
	521L	++ 100	++ 100	+ 100	+++ 50	+ 100	++ 100	+ 100	+++ 50
	524C	+++ 1600	+++ 1600	++ 1600	+++ 1600	++ 1600	+ 3200	— 50	— 50
521L	204D	++ 50	++ 50	+ 50	+ 50	++ 50	+++ 50	++ 400	++ 800
	519F	+++ 50	++ 50	++ 50	++ 50	++ 50	+ 100	+ 100	+ 200
	520A	— 40	— 40	— 40	— 40	— 40	— 40	+ 800	++ 400
	521L	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50
	524C	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50
524C	204D	++ 50	++ 50	+ 50	+ 50	++ 50	++ 50	++ 800	++ 800
	519F	++ 50	+ 50	— 50	— 50	— 50	— 50	+ 100	+ 200
	520A	+++ 50	+ 50	— 50	++ 50	++ 50	— 50	++ 100	++ 400
	521L	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50
	524C	— 50	— 50	— 50	— 50	— 50	— 50	— 50	— 50

Table 9 shows the complete reciprocal removal of agglutinins in all antisera except that prepared against *Phytomonas vignae* 521L and *Ph. syringae* 524C. The results obtained by three absorptions with *Phytomonas cerasi* 520A, *Ph. tabaci* BBF, *Ph. angulata* 410, *Ph. primulae* 519F, are comparable to those obtained by three absorptions with the strains of *Pseudomonas fluorescens* 204D and $80F_2$.

The results presented in table 9 indicate that the protein fractions of *Phytomonas tabaci* BBF, *Ph. angulata* 410, *Ph. primulae* 519F, *Ph. cerasi* 520A and *Pseudomonas fluorescens* 204D and $80F_2$ are antigenically identical. The data cannot be said to indicate such antigenic identity with respect to *Phytomonas syringae* 524C and *Ph. vignae,* but it likewise cannot be said with certainty in the case of these two strains that all cells used in the production of antisera and as antigens in agglutination reactions were in the "S" state. Failure of reciprocal agglutinin absorption to indicate identity in these cases had rather been expected because of the tendency of variant "M" forms to appear in any cultivation of these strains apart from homologous antisera. That this accounts for the failure to demonstrate equivalent reciprocal agglutinin absorption in tests of these strains with the other members of the group receives additional confirmation in a study of the absorptive capacity of *Phytomonas vignae* 521L. In what approximated the "S" phase in the case of this species all agglutinins were removed from each of the other sera, however, the other organisms with the exception of *Phytomonas syringae* 514C failed to remove agglutinins from *Phytomonas vignae* 521L antiserum to the equivalent extent. This can only be accounted for on the assumption that the so-called "S" phase of *Phytomonas vignae* 521L contained all of the antigens possessed by *Phytomonas tabaci* BBF, *Ph. angulata* 410, *Ph. cerasi* 520A, *Ph. primulae* 519F and *Pseudomonas fluorescens* 204D and $80F_2$ in the "S" phase but, in addition, some other antigenic fraction was present such as could be accounted for by the presence of capsular material due to the "M" variants which always appeared in the cultivation of the organism.

Variation in antigenicity of green fluorescent bacteria.—The foregoing experiments on a number of green fluorescent bacteria have indicated that a close relationship exists between the bacterial pathogen *Ph. tabaci* and the soil saprophyte *Ps. fluorescens.* The concept that one and the same organism may at one time be parasitic and another time saprophytic is not at all unusual in the light of our knowledge of bacterial variation. Studies dealing with bacterial variation have shown *(11)* that numerous bacterial pathogens undergo temporary variations as a result of change in environment. Such adaptations frequently include correlated variations in which the colony morphology, virulence, and antigenic characters of the cells vary. In this study as shown on the following pages similar alterations in antigenic structure with a correlated variation with respect to virulence have been noted.

It is not feasible to discuss the subject of bacterial variation in this paper. Reference has been made to an extensive review *(11)*. Certain workers *(2)* question the value of serological reactions as a means of measuring relationship between plant pathogens while others *(22)* believe a definite positive correlation exists. The authors realize the many pitfalls confronting workers in this field of study but believe that the earlier conclusions drawn from investigations which did not take into consideration dissociation phenomena or separate the different bacterial phases for study are not valid objections at the present time. Recent reports *(20)* stating the lack of relation between pathogenicity and agglutination phenomena have apparently erred in a similar manner in disregarding the phase study of the cultures employed.

The authors consider that serological techniques measure the sum of the morphological and physiological characteristics (including pathogenicity) of the germ plasm of bacterial cells. Such techniques are a measure of the chemical composition of cells or the chemical composition of the surface areas of bacterial cells. It is well established that numerous groups of organisms in the "M" phase have slightly different morphological, physiological, and serological characteristics. However, if the surface covering of polysaccharide material is removed from these different groups, the remaining "S" cells are identical. The polysaccharide surface covering of such "S" cells is known to be a product of the cell environment. Pathogenicity is just as much a variable factor as other physiological and morphological characteristics of microorganisms. In many diseases of man and other animals such as typhoid fever, pneumonia, "Bangs" disease, pullorum etc., serological analysis is employed as the final and especially sensitive test indicating the presence of the bacteria responsible for the disease. So proved and accepted is this method of serological techniques that in no case is it required upon obtaining a positive serological result to inject a second person with a typhoid or other disease organism and produce the disease to be certain that the first person in reality had typhoid fever. It is well known that many pathogens maintained for any prolonged period on laboratory culture media become avirulent. In some instances loss of virulence occurs slowly and at other times fairly rapidly. It is also known that virulence may be enhanced when the microorganism is in a suitable environment or host. It is known that in epidemics of disease the virulence of the microorganism varies from one epidemic to another. All gradations of virulence from a practically complete avirulent form to a form of maximum virulence may exist at different times. Wolfe and Foster in giving the name wildfire to this leafspot disease of tobacco may have inadvertently described how rapidly normal soil and plant forms may become pathogenic under suitable conditions. Since pathogenicity is a relative term and must be considered in relation to host susceptibility; bacterial variation of plant pathogens should of necessity take into consideration the physiology and environment of the host plant.

It should not be assumed that since *Phytomonas tabaci* and *Ps. fluorescens* in the "S" form are identical that they are at all times equally pathogenic. The pathogenicity of a microorganism as stated above is determined equally by the environment or host of the bacterial cell and by the inherent nature of the organism itself. The authors are therefore presenting this new concept that *Ph. tabaci* and *Ps. fluorescens* are identical inherently and that the harmless, relatively avirulent organism *Ps. fluorescens* under suitable conditions can readily increase in virulence until it possesses all the characteristics of the pathogen known as *Ph. tabaci. Pseudomonas fluorescens* therefore is considered a potential hazard in that under suitable environmental conditions, this form builds a characteristic polysaccharide material which makes it similar in all respects to *Ph. tabaci.*

The poor titres obtained with cells of *Pseudomonas fluorescens* 353, table 4, in agglutination tests using antisera prepared against *Pseudomonas fluorescens* 204 and *Phytomonas tabaci* BB led to a further investigation of this strain. This culture, 353, was used as an antigen in animal inoculation and the antiserum obtained was tested against cells of *Pseudomonas fluorescens* 204 and found to react well, 1 to 6400, although cells of 353 agglutinated only in dilutions of 1 to 50 and 1 to 100 in antiserum prepared against 204. Two attempts were made to change the antigenic structure of 353. One transfer of 353 in nutrient broth containing 10 per cent of homologous immune serum resulted in cells which reacted with 204 antiserum in a dilution of 1 to 6400. Five serial transfers in tobacco extract broth resulted in cells of strain 353 which reacted with 204 antiserum in a dilution of 1 to 3200. It would appear that some slight capsular difference had served to mask the antigenic similarity of 353 to other members of the group. Table 10 shows this in tabular form.

That similar slight antigenic changes constantly occur in nature is suggested by a study of members of *Pseudomonas fluorescens* isolated at frequent intervals from growing clover representing young to mature plants. Agglutination reactions of a few of these isolates showing the general trend as the plant approaches maturity are presented in table 11. Culture 353 was isolated from the fertile soil early in the spring. Culture 230 was isolated from the epiphytic flora of a very young clover plant. Culture 248 was isolated from a half grown clover plant and cultures 204 and 504 were isolated from mature plants.

Table 10.—Effect of various environmental conditions on the agglutinability of Pseudomonas fluorescens.

	PS. FLUORESCENS 353 ANTI-SERUM DILUTIONS									PS. FLUORESCENS 204 ANTI-SERUM DILUTIONS								
BACTERIA	1-50*	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800*	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800
Ps. fluorescens 204	4†	4	4	4	4	2	–	–	–	4	4	4	4	4	4	4	4	2
Ps. fluorescens 353	4	4	4	4	4	4	4	2	–	4	2	–	–	–	–	–	–	–
Ps. fluorescens 353 AS‡	4	4	4	4	4	4	4	3	1	4	4	4	4	4	4	4	3	1
Ps. fluorescens 353 TE§	4	4	4	4	4	4	2	–	–	4	4	4	4	4	4	4	2	–

* Serum dilutions—higher dilutions and saline controls were negative.
† Numbers refer to degree of macroscopic agglutination. 4 = complete; 1 = slight; – = negative.
‡ 353 AS = after growth in homologous anti-serum.
§ 353 TE = after growth in unheated tobacco extracts.

Table 11 shows that the antiserum prepared against *Phytomonas tabaci* BB either failed to react or at best reacted only to a slight degree with isolates obtained early in the life of the plant. Week by week antigentic similarity became more pronounced until at the end of the season the typical member of *Pseudomonas fluorescens* isolated from the mature plant proved antigenically identical with *Phytomonas tabaci* BB in the "M" phase. This suggests that the capsular antigenicity of *Phytomonas tabaci* is a reflection of continued reproduction as an epiphyte.

Table 11.—Agglutination reactions of progressive isolations from clover of Pseudomonas fluorescens with anti-serum of Phytomonas tabaci BB.

	SERUM DILUTIONS							
BACTERIA	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400*
Ps. fluorescens 353	4†	2	–	–	–	–	–	–
Ps. fluorescens 230	–	–	–	–	–	–	–	–
Ps. fluorescens 248	4	4	4	3	1	–	–	–
Ps. fluorescens 204	–	2	4	4	4	4	2	
Ps. fluorescens 504	4	4	4	4	4	4	4	2

* Higher dilutions and saline control were negative.
† Numbers refer to degree of macroscopic agglutination, 4 = complete, 1 = slight, – = negative.

DISCUSSION AND APPLICATION OF RESULTS

Practical value of the antigenic identity found to exist between Ph. tabaci and Ps. fluorescens.— In view of the results previously obtained showing the close similarity and identity of *Ph. tabaci* and *Ps. fluorescens* it was realized that a serological method was available for determining the presence of leafspot organisms in soils and on normal leaves as well as on diseased plants. The agglutination technique described under the section on methods and as employed in the earlier part of this bulletin was used to detect the presence of leafspot bacteria in seedbed soils and on normal plants. Such a method can be readily employed in detecting the presence of leafspot organisms in seedbed soils before and after steaming, in the field and on tobacco and other plants which may not be diseased.

Influence of steaming on microorganisms in tobacco seedbeds.— Although the steaming of tobacco seedbeds has been recommended by almost every writer on the subject as a means of eliminating wildfire and angular leafspot organisms from the soil, there has been found in the literature no record of any serious study of the effects of soil steaming upon these organisms. The authors began an investigation of this phase of the subject in the spring of 1937 in cooperation with the late Otto Olson of the United States Department of Agriculture and have continued this study through 1938, 1939, and 1940. In the course of the investigation a large number of soil samples and seedlings from Lancaster county seedbeds were collected and examined for bacteria by the methods previously described.

Table 12 shows the numbers of bacteria, fungi, and green fluorescent organisms obtained per gram of soil and the presence of wildfire microorganisms in 20 representative soils samples collected in April and May, 1940, from seedbeds before steaming.

The same seedbeds after steaming in accordance with the sanitary recommendations made for the control of tobacco wildfire were again examined. Not more than four beds represent the work of one operator. The results of the bacteriological analyses of the same 20 seedbeds shown in table 12 after steaming are shown in table 13.

It is shown in table 13 that the wildfire organism was found present in all 20 seedbed soils following the steam treatment. This indicates that the steaming of seedbed soils as practiced in the district does not result in sterilization as the common terminology, "seedbed sterilization", has led many to believe. It does not result in the destruction of certain of the less resistant bacteria and con-

Table 12.—Numbers of microorganisms in Lancaster county seedbed soils before steam treatment.

FARM NUMBER	ORGANISMS PER GRAM OF SOIL			
	Bacteria	Fungi	Green fluorescent bacteria	Wildfire organisms
1	11,000,000	45,000	100	Present
2	34,000,000	90,000	200	Present
3	12,000,000	47,000	50	Present
4	9,500,000	5,000	100	Present
5	8,500,000	22,000	10	Present
6	7,600,000	35,000	100	Present
7	17,000,000	41,000	50	Present
8	19,000,000	10,000	200	Present
9	24,000,000	85,000	100	Present
10	65,000,000	180,000	200	Present
11	22,000,000	90,000	100	Present
12	38,000,000	75,000	100	Present
13	18,000,000	34,000	100	Present
14	29,000,000	37,000	200	Present
15	16,000,000	21,000	50	Present
16	13,000,000	9,500	10	Present
17	27,000,000	23,000	100	Present
18	31,000,000	32,000	100	Present
19	19,000,000	16,000	50	Present
20	8,100,000	13,000	10	Present

sequently can not be compared to the pasteurization process used in dairy and food industries. The data supplied in table 13 supports the observations of Chapman and Anderson *(4)* who noted that seedbed steaming had either little or no relation to the occurrence of wildfire in the field. Attention is directed to the fact that the data presented in table 13 represent in some cases analyses made immediately after soil steaming, while in other cases the samples were taken several days following steaming. In the latter instances some recontamination of the bed may have occurred prior to sampling, but in the case of beds on farms 1, 2, 10, 11, and 15 any significant recontamination prior to sampling appears unlikely.

The examination of more than 200 seedbed soils failed to reveal a single case in which steaming resulted in the absence of green fluorescent bacteria. It will be noted that in those cases where sampling was done immediately following steaming, a reduction in the number of these organisms was found to have taken place during the steam treatment. This reduction, however, was not great enough in most cases to suggest that complete destruction could be accomplished by any reasonable increase in the duration of the treatment. This conclusion was substantiated in an experimental test. Steaming for a period of 1 hour failed to destroy all green fluorescent organisms in the upper 4 inches of soil.

Table 13.— Numbers of microorganisms in Lancaster county seedbed soils following steam treatment; analysis of the same seedbeds as were reported in table 12.

FARM NUMBER	INTERVAL BETWEEN STEAMING AND SAMPLING	ORGANISMS PER GRAM OF SOIL			
		Bacteria	Fungi	Green fluorescent bacteria	Wildfire organisms
1	1 hour	350,000	60	10	Present
2	2 hours	1,400,000	175	100	Present
3	1 week	1,500,000	450	200	Present
4	2 weeks	1,200,000	200	200	Present
5	3 weeks	2,100,000	5,000	200	Present
6	3 weeks	1,800,000	40,000	100	Present
7	3 weeks	3,900,000	5,000	100	Present
8	3 weeks	2,400,000	200	100	Present
9	1 week	3,100,000	450	200	Present
10	1 day	2,800,000	300	100	Present
11	4 hours	650,000	70	50	Present
12	2 weeks	9,500,000	30,000	200	Present
13	3 weeks	19,000,000	70,000	200	Present
14	2 days	2,600,000	100	50	Present
15	2 hours	970,000	40	10	Present
16	4 days	3,200,000	800	10	Present
17	2 weeks	11,000,000	9,000	200	Present
18	2 days	1,700,000	200	10	Present
19	3 weeks	9,400,000	6,500	100	Present
20	2 weeks	3,500,000	5,900	100	Present

In any consideration of the effects of steaming it should be borne in mind that it is much more difficult to effect destruction of microorganisms in a heavy clayey soil than in a light sandy soil. Consequently the average Lancaster county grower could not expect the destructive effect which may be obtained on the lighter soils of certain other tobacco districts. Even the reaction of the soil of Lancaster seedbeds is unfavorable from the standpoint of disinfection. Acid soils are more easily sterilized than are natural soils and very few seedbeds have been found in the Lancaster area with a pH value below 6.8.

Increase in bacterial numbers following steaming.—Table 13 shows that in those cases in which the second samples were taken one to three weeks after the steam treatment, there appears to have been an actual increase in the number of green fluorescent bacteria in the seedbed soils. In order to determine whether such increases in the number of microorganisms was a characteristic change following steaming, the seedbeds of six Lancaster county farms were examined over a 7-week interval of time for bacteria, fungi, and the presence of green fluorescent forms. From 10 to 30 isolations of green fluorescent bacteria were made from each seedbed sample, and by means of the agglutination test, using antisera prepared with known strains of the wildfire organism, the percentage of this type of green fluorescent bacteria was determined. The data are presented in table 14.

Table 14.—Results of interval examination of six typical seedbeds in Lancaster county.

FARM NUMBER	SAMPLING DATE	ORGANISMS PER GRAM OF SOIL			
		Bacteria	Fungi	Green fluorescent bacteria	Wildfire organisms*
		number	*number*	*number*	*per cent*
1	April 17	1,800,000	40,000	100	17
	May 16	138,000,000	95,000	200	33
	June 4	670,000,000	80,000	200	70
2	April 17	3,900,000	5,000	100	10
	May 16	110,000,000	70,000	200	67
	June 4	980,000,000	40,000	200	82
3	April 17	3,000,000	450	50	20
	May 16	110,000,000	30,000	100	17
	June 4	1,200,000,000	400,000	200	45
4	April 17	19,000,000	25,000	100	40
	May 16	105,000,000	40,000	100	55
	June 4	430,000,000	3,000	200	75
5	April 17	2,400,000	200	10	15
	May 16	115,000,000	4,000	50	10
	June 4	900,000,000	30,000	100	30
6	April 17	650,000	60	10	50
	May 16	240,000,000	55,000	200	17
	June 4	870,000,000	38,000	100	60

* Wildfire organisms present expressed as percentages of isolations of green-fluorescent bacteria found identical with the wildfire organism on the basis of serological tests.

It is to be noted in table 14 that during the period of seven weeks intervening between the first and third samplings, there was a considerable increase in both bacteria and fungi. Among the types of bacteria taking part in this reproduction were the green fluorescent organisms. Of more importance, however, the data indicates an increase in that part of the green fluorescent flora that is associated with the bacterial leafspots of tobacco. These data show that not only are there more green fluorescent bacteria in the typical steamed seedbed some seven weeks after seeding than are present at the time of seeding but that the percentage of these organisms which can be identified as wildfire or angular leafspot bacteria actually increases during this period. These data may serve to explain the observations of Clinton and McCormick *(6)* that steamed beds had a greater amount of wildfire than unsteamed beds. Inasmuch as all of these beds contained seedling tobacco, the possibility exists that this reproduction may have taken place on the plants rather than in the soil and that the organisms were washed into the soil in the watering of the beds. Other experimental evidence, however, indicates that at least a part of this increase in numbers of leafspot organisms actually took place in the soil as a substrate. Well-manured soil was placed in pots and steamed for 20 minutes. Following this heat treatment the wildfire organism was added and counts were made at intervals over a period of three weeks, using the same methods employed previously. No crop was grown in this experiment but the results showed that an actual increase in numbers of the wildfire organism had occurred during the three weeks period, although many sporeformers and other microorganisms were present as competitors.

It is worthy of note in connection with the data presented in table 14 that bordeaux mixture was used at intervals on all six beds.

It may be concluded therefore that the mere presence of the leafspot organism in seedbed soils has not been the determining factor leading to wildfire and that some other factor or factors have been responsible for the occurrence of leafspot diseases of tobacco.

Bacterial flora of the tobacco plant.—During the course of this study the bacterial flora of hundreds of tobacco samples from both the seedbed and the field was determined. Data collected in 1937 did not include serological analyses of the green fluorescent bacteria encountered on the plant. Beginning in 1938, however, the green fluorescent isolates obtained from such plant material were subjected to agglutination studies and the percentages of leafspot organisms were determined. A summary of the data obtained showing the incidence of the types of microorganisms encountered is shown in table 15.

It is to be noted in table 15 that the three gram-positive types encountered appear to compose a very stable portion of the bacterial flora of the leaf, while the gram-negative types, on the other hand, were found to vary considerably. The leafspot organisms formed a portion of the normal flora of almost all tobacco plants.

It was found that the percentages of leafspot organisms in the green fluorescent flora may vary from 0 to 100 per gram in the case of very young plants, but that during the course of the growing season the percentages rise steadily until practically all green fluorescent organisms on a plant in the latter part of the season may be wildfire and angular leafspot in nature. This increase was found to be directly associated with the extent of the reproduction

Table 15.—Bacterial flora of Pennsylvania tobacco in seedbed and field.

TYPE OF BACTERIA	STABILITY OF POPULATION	NUMBERS USUALLY FOUND PER GRAM
Cocci, gram-positive, both chromogenic and non-chromogenic	Stable	Less than 10,000
Rods, gram-positive, non-spore-forming	Stable	Less than 10,000
Rods, gram-positive, spore-forming	Stable	Less than 75,000
Rods, gram-negative, non chromogenic	Variable	From less than 1,000 to more than 1,000,000
Rods gram-negative, pink pigment	Variable	From very few to several million
Rods, gram-negative, yellow pigment	Variable	From a few thousand to a few million
Rods, gram-negative, green-fluorescent pigment	Variable	From very few to 20,000,000

of the green fluorescent bacteria on the plant. In other words, the greater the number of generations of green fluorescent bacteria upon the plant, the greater is the percentage of forms which can be identified as leafspot organisms. Upon the aerial portions of these plants, the leafspot organisms reproduce in accordance with climatic conditions and host nutrition, and only under conditions of lowered host resistance does the association become detrimental to the green plant. At such times the green fluorescent forms fluctuate in number. The conditions which were associated with this fluctuation are shown in table 16.

Table 16 shows that if the relative humidity has been low, as is often the case in the summer, the bacterial population of tobacco leaves will usually be found to be less than 100,000 per gram, with the stable gram-positive portion of the flora comprising the greater part of the count. Under such conditions it is often necessary to utilize enrichment cultivation on selective media to demonstrate the presence of the gram-negative types. On the other hand, high relative humidity in seedbed or field is followed by a rapid rise in the gram-negative flora of the leaf. The particular type or types of gram-negative bacteria which reproduce most abundantly under such conditions appears to depend upon the nutrition and physiology of the tobacco plant. Under satisfactory conditions of nutrition and environment, saccharolytic yellow rods play the major role in this gram-negative development, while under conditions of high nitrogen nutrition, proteolytic yellow rods and green fluorescent bacteria reproduce so rapidly as to obscure the other forms. A violent upset of the microbial equilibrium in the direction of either proteolytic yellow or green fluorescent forms was found by the authors to be indicative of unfavorable environmental conditions in the seedbed or the field.

Table 16.—Factors associated with the relative numbers of the several types of organisms which comprise the normal flora of the growing tobacco plant.

TYPE OF BACTERIA	GENERAL REMARKS	CONDITIONS ASSOCIATED WITH DOMINANCE
Gram-positive organisms, rods and cocci	Dominant type in cases of counts below 100,000 per gram. Presence demonstrated by desiccation of sample in cases of counts much above 100,000.	The characteristic flora of tobacco following periods of low humidity.
Gram negative rods, non-chromogenic	Includes representatives of *Proteus, Aerobacter,* and *Achromobacter.* A few can be demonstrated on most samples. Rarely dominant on Pennsylvania tobacco.	The characteristic flora of a certain type of tobacco not grown in Pennsylvania. Found on some Pennsylvania tobacco following periods of high humidity.
Gram-negative rods, pink pigment	Can usually be demonstrated on Pennsylvania tobacco but rarely in large numbers.	Characteristic of certain inferior tobaccos of the flue-cured type.
Gram-negative yellow pigment (a) Saccharolytic type	These peritrichic rods can be demonstrated on any tobacco of good quality and when dominant the counts range from 100,000 to 1,000,000.	This is the typical flora of good tobacco of most types following periods of moderate to high humidity.
(b) Proteolytic type	These monotrichic rods can be demonstrated on most tobacco. When dominant the counts range from 500,000 to 3,000,000	This is characteristic of certain tobacco with unbalanced nutrition following periods of high humidity.
Gram-negative rods, green fluorescent pigment	These lophotrichic rods can be found on almost all samples of growing tobacco. When dominant the counts range from 500,000 to 20,000,000. High counts often associated with high counts of proteolytic yellow rods and non-chromogenic gram-negative rods.	This is the typical flora of tobacco with high nitrogen and low potassium uptake following periods of moderate to high humidity.

Among the agricultural plants subjected to study by the authors in their efforts to establish the nature of the normal bacterial flora of living plants, red clover received considerable attention. From the first it was apparent that the flora of the normal red clover plant closely resembles the flora of the normal tobacco plant. The same stable population of gram-positive organisms was found present; the same saccharolytic yellow rods flourished in periods of moderate to high humidity. More important from the standpoint of this research, green fluorescent rods were found to be a normal part of the flora of the clover plant and were found to appear in great numbers under conditions similar to those inciting such reproduction on the tobacco plant. Most important of all, however, it was found that this green fluorescent flora of mature clover plants could be identified as the organisms of wildfire and angular leafspot, while on the other hand the green fluorescent flora of very young clover proved serologically different from known wildfire and angular leafspot bacteria. In other words the antigenic nature of isolates from young clover was found to be quite different from that of the leafspot organisms, while the antigenic nature of isolates from old clover proved identical to that of the leafspot organisms.

In order to determine whether the microorganisms of leafspot diseases could survive as free living forms in the soil, their ability to utilize various simple substrates to meet their nutritional requirements was examined. Four known cultures associated with wildfire and angular leafspot, 1. *Ph tabaci* No. 591[1]; 2. *Ph. tabaci* No. 12; 3. *Ph. tabaci* No. 532; and 4. *Ph. angulatum* No. 410, were inoculated into seven simple culture media. The ability of these organisms to grow upon repeated subculture in these media is shown in table 17.

Table 17 shows that all four leafspot organisms were able to grow on these seven substrates with the exception of the nitrated coal medium. Very few disease causing organisms possess the ability to grow on such simple substrates. This fact emphasizes the unusual ability of the green fluorescent bacteria associated with leafspot disturbances to grow readily in an environment less suitable than is commonly encountered in the soil. These data also indicate that leafspot organisms could readily live from year to year in soil even in the absence of tobacco refuse.

The evidence presented indicates that the organisms of wildfire and angular leafspot are widely distributed in nature and occur normally on many plants as harmless saprophytes.

---

[1] The authors wish to express their thanks to W. S. Beach for cultures No. 591 and No. 12; E. E. Clayton for culture No. 532 and J. Johnson for No. 410.

Table 17.—Growth of wildfire and angular leaf spot organisms on various substrates.

	ORGANISM			
SUBSTRATE	Wildfire No. 591	Wildfire No. 12	Wildfire No. 532	Angular Leaf spot No. 410
Glucose-asparagin mineral salts	Excellent	Excellent	Excellent	Excellent
Glucose-ammonium sulfate-mineral salts	Good	Fair	Good	Good
Asparagin-mineral salts	Excellent	Good	Excellent	Good
Malic acid-ammonium sulfate-mineral salts	Good	Good	Good	Good
Succinic acid- ammonium sulfate-mineral salts	Good	Fair	Good	Fair
Crude petroleum-ammonium sulfate-mineral salts	Fair	Poor	Fair	Fair
Nitrated Coal solution-ammonium sulfate-mineral salts	Fair	None	Poor	Poor

ANTIGENIC RELATIONSHIPS

The results of these investigations have demonstrated that the leafspot organism is always present in soil and that *Phytomonas tabaci* is identical morphologically, culturally, and serologically with the common soil form *Pseudomonas fluorescens*. This is a new concept in that it was formerly held that *Phytomonas tabaci* was a parasitic form which did not live independently of its host and survived over winter in tobacco sheds on old tobacco, etc. The complete reciprocal agglutinin absorption which was demonstrated in both the Dawson "M" and "S" phases between culture BBF a virulent culture of *Phytomonas tabaci* and *Pseudomonas fluorescens* 204D a common soil bacterium leaves little if any doubt, that the two forms are identical. The value of serological analyses in showing species relations as well as the alteration occurring in virulence and antigenic characters in bacterial variation is well established.

The question naturally arises as to how a normal soil bacterium such as *Pseudomonas fluorescens* will suddenly become involved in a disease which spreads so rapidly that the name, wildfire accurately describes it. The question may be answered by a consideration of the ecological relation existing between the tobacco plant and the bacterium. In nature it is characteristic that an approximately balanced condition exists between contending species. Such a balance is apparent in healthy tobacco where leafspot organisms are present on the leaf in fairly large numbers, as well as being present in the soil. Any factor which disturbs this balance could lead to an altered relationship between the plant and its normal bacterial flora. Such disturbances with resulting leafspot troubles were recognized and described long before the serious disease outbreaks of 1917 *(13 and 15)*. The relationship of such disturbances to leafspot diseases were emphasized by investigators of the 1917 epidemics *(8 and 23)*. Similar findings of the authors of this paper will appear in subsequent publications.

Although these studies indicate that the peculiar antigenic components which characterized the "M" phase of *Phytomonas tabaci* are enhanced by reproduction of the organism as a plant epiphyte, these studies do not indicate that such progressive changes in the nature of the organism lead to a degree of virulence capable of overcoming the resistance of a normal plant. Strain 80 used in this experimental work was isolated from a normal, healthy, tobacco plant which showed no evidence of disease at any time during the season although other plants grown under less favorable conditions in nearby plots were seriously damaged. Strain 80, it may be added, was found to be more virulent on a susceptible host than any strain procured from other investigators.

These studies have shown not only the very close relationship that exists between *Phytomonas tabaci* and *Pseudomonas fluorescens* but also that there is little if any distinction between other plant pathogens such as *Ph. primulae, Ph. cerasi, Ph. vignae, Ph. syringae,* and *Pseudomonas fluorescens.* The demonstration of the relationship between these numerous forms indicates the fallacy of considering and naming an organism a distinct species because of its ability to attack different hosts. Such a procedure is not followed with tuberculosis bacteria. Cattle, hogs, man, and fowl are all susceptible to tuberculosis but the organisms causing the disease in these four different animal groups are recognized as being practically identical.

SUMMARY

In a study of a large number of green fluorescent gram-negative bacteria isolated from seedbed soils as well as plant material from seedbed and field which were compared with known cultures, the authors feel that the following conclusions are warranted at this time from the data obtained:

1. An extensive serological study employing single-cell isolations indicates that the organism associated with the leafspot disease known as wildfire is identical antigenically with the common saprophyte, *Pseudomonas fluorescens.*
2. From serological studies it is concluded that *Phytomonas angulata, Ph. primulae* and *Ph. cerasi* are also identical with the saprophyte *Pseudomonas fluorescens.* Due to the inability to maintain cultures of *Phytomonas vignae* and *Ph. syringae* in the "S" phase, it has not been possible to show the identity of these with *Pseudomonas fluorescens*, but the data obtained suggest the possibility of such identity.

3. A study of the transformation of the capsular antigenicity of *Pseudomonas fluorescens* on normal clover plants permits the conclusion that the antigenic specificity of the wildfire organism in the "M" phase is a reflection of capsular changes occurring during the reproduction of the organism as a harmless epiphyte.
4. Seedbed steaming as practiced in Lancaster county does not result in the elimination of the bacteria associated with the leafspot diseases of tobacco. On the contrary these organisms are more prevalent following steaming than immediately prior thereto.
5. Tobacco in the Lancaster area is produced as a rule in the presence of leafspot organisms, inasmuch as the organisms associated with leafspot diseases have been found in all seedbeds examined and on most plant material from seedbed and field.
6. Inasmuch as the organisms have been found on most normal tobacco as well as on diseased plants, the appearance of the disease can not be attributed to the mere presence of the organisms but to some other factor or factors.

BIBLIOGRAPHY

(1) Bergey, D. H. et. al. 1939. Bergey's Manual of Determinative Bacteriology. 5th edition. *Williams and Wilkins Co., Baltimore, Md.* pp. 124-218.

(2) Burkholder, W. H. 1937. Serological reactions for the determination of bacterial plant pathogens. *Phytopath.* **27**: 572-74.

(3) Castellani, A. 1902. Die Agglutination bei gemischter Infektion und die Diagnose der Letzteren. *Zeitsch. f. Hyg. u. Infektionskr.* **40**: 1.

(4) Chapman, G. H. and P. J. Anderson. 1921. Tobacco wildfire. *Mass. Agr. Expt. Sta. Bul.* **203**.

(5) Clayton, E. E. 1936. Water soaking of leaves in relation to development of the wildfire disease of tobacco. *Jour. Agr. Tec.* **52**: 239-269.

(6) Clinton, G. P. and F. A. McCormick. 1922. Wildfire of tobacco in Connecticut. *Conn. Agr. Expt. Sta. Bul.* **239**.

(7) Fromme, F. D., and S. A. Wingard. 1922. Blackfire or angular leafspot of tobacco. *Va. Agr. Expt. Sta. Tech. Bul.* **25**.

(8) Fromme, F. D., and S. A. Wingard. 1922. Blackfire and wildfire of tobacco and their control. *Va. Agr. Expt. Sta. Bul.* **228**.

(9) Fromme, F. D. and T. J. Murray. 1919. Angular leafspot of tobacco, an undescribed bacterial disease. *Jour. Agric. Res.* **16**: 219-228.

(10) Gruber, M. and H. E. Durham. 1896. Eine neue Methode zur raschen Erkennung des Choleravibrio und des Typhusbacillus *München. med. Wchnschr.* **43**: 285.

(11) Hadley, P. 1937. Further advances in the study of microbic dissociation. *Jour. Inf. Dis.* **60**: 129-192.

(12) Harris, R. G. 1940. A serological study of the monotrichous members of the Genus *Pseudomonas*. Ph.D. dissertation. *The Central Library, The Pennsylvania State College, State College, Pa.*

(13) Johnson, J. 1914. The control of diseases and insects of tobacco. *Wis. Agr. Expt. Sta. Bul.* **237**.

(14) Julianelle, L. A. 1936. Immunological specificity of *Bacterium aerogenes* and its antigenic relation to pneumococcus Type II and Friedlander's bacillus, Type B. *Jour. Immunol.* **32**: 21-34.

(15) Killebrew, J. B. 1880. Report on the culture and curing of tobacco in the United States, *Report on Production of Agriculture, Tenth Census, 1880.*

(16) Krumwiede, C., G. Cooper, and D. J. Provost. 1925. Agglutinin Absorption. *Jour. Immunol.* **10**: 55-239.

(17) Lehmann, L. B. and R. O. Neumann. 1931. Determinative Bacteriology (7th edition translated by Breed). **2**: 466-483. *G. E. Stechert & Co., New York City.*

(18) McMurtrey, J. E., Jr. 1938. Symptoms on field grown tobacco characteristic of the deficient supply of each of several essential chemical elements. *U. S. D. A. Tech. Bul.* **612**.

(19) McMurtrey, J. E., Jr., et al. 1934. Fertilizer tests with tobacco. *Md. Agr. Expt. Sta. Bul.* **358**.

(20) McNew, G. L. and Braun, A. C. 1940. Agglutination test applied to strains of *Phytomonas stewarti*. *Bot. Gaz.* **102**: 64-78, also pp. 78-89.

(21) Naghski, J. 1941. A serological study of some lophotrichic members of the green fluorescent bacteria. Ph.D. dissertation. *Central Library, The Pennsylvania State College, State College, Pa.*

(22) Stapp, C. 1935. Contemporary understanding of bacterial plant-diseases and their causal organisms. *The Bot. Rev.* **1**: 405-25.

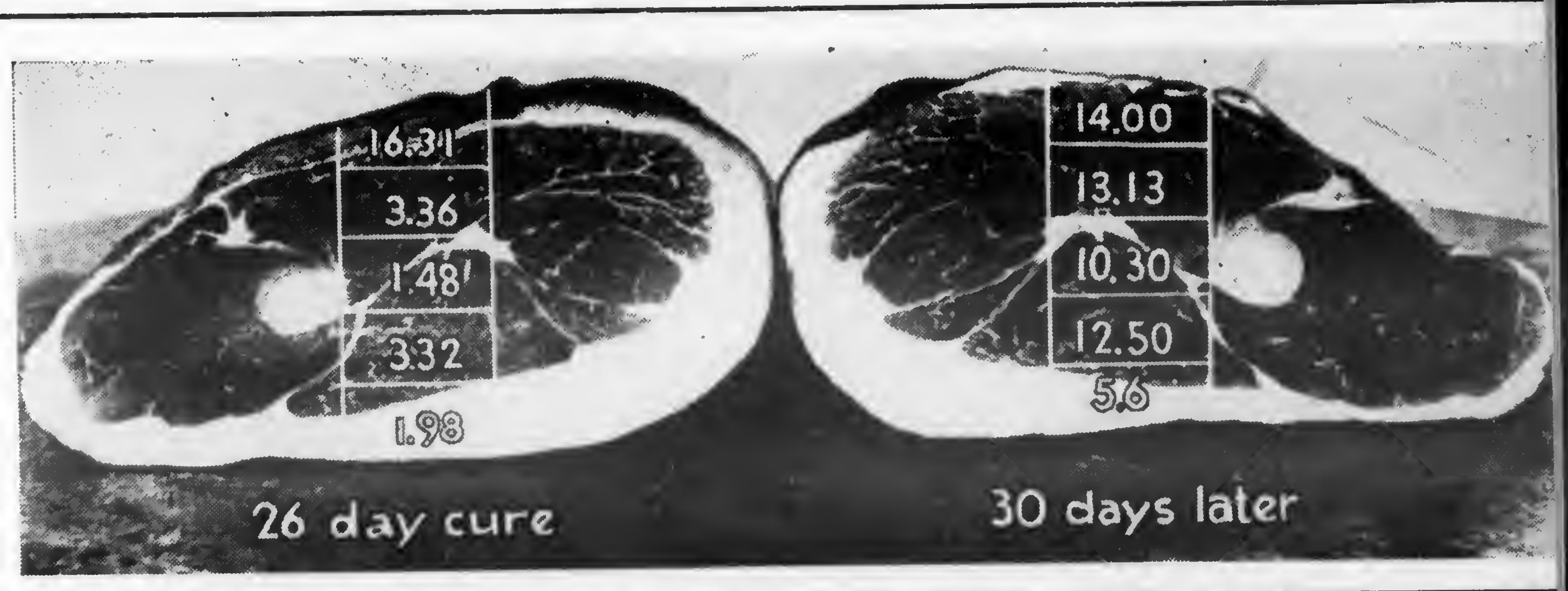
(23) Wolf, F. A. 1922. Wildfire of tobacco. *N. Car. Agr. Expt. Sta. Bul.* **246**.

(24) Wolf, F. A., and A. C. Foster. 1918. Tobacco wildfire. *Jour. Agr. Res.* **12**: 449-458.

(25) Wright, W. H., and E. F. McCoy. 1927. An accessory to the Chambers apparatus for the isolation of single bacterial cells. *Jour. Lab. and Clin. Med.* **12**: 795-800.

(26) Wright, W. H., and H. Nakajima. 1929. The growing of pure cultures from single cells of non-spore forming bacteria. *Jour. Bact.* **17**: 10.

BULLETIN 427 **APRIL, 1942**



Effect of aging on distribution of salt in cured hams. Test pieces were cut out of the hams as shown by the white lines. The figures are percentages of salt in the samples at the end of the 26-day cure and 30 days later.

Methods of Hastening
ABSORPTION OF SALT IN FARM-CURED HAM

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

Methods of Hastening Absorption of Salt In Farm-cured Ham*

P. T. ZEIGLER, and R. C. MILLER†

THE primary purpose of quick ham curing is to hasten introduction of salt into all of the cell tissues to eliminate spoilage as a result of souring. Other benefits of economic importance are quicker turnover and less shrinkage. The palatability of short-cure products as compared to longer-cure is a matter of personal opinion. Short cures do not permit enzyme action to develop flavors that some people value highly in cured pork. However, subsequent ageing of the ham will bring these about, providing the salt content is sufficiently high to carry the ham through such a period. Packers are frank to admit that "quickies" (short-cure hams) should be used within three months.

The experimentation herein reported was conducted to determine the procedure that the farmer might follow successfully, in not only hastening the cure, but in preserving the fresh product for future curing. This work supplements previous curing work reported in Bulletin 355, "Rate of Salt Absorption in Curing Pork."

EXPERIMENTAL PROCEDURE

The curing formula employed was 8 pounds of salt, 3 pounds of granulated, brown sugar or cerelose, and 3 ounces of saltpeter (KNO_3). When dissolved in 4 gallons of water this formula produces pickle of approximately 85° salinity and in 5 gallons of water about 75° salinity as measured by a salimeter. Sodium nitrite ($NaNO_2$), which is commonly used to replace a small part of the saltpeter (KNO_3) or sodium nitrate ($NaNO_3$) in producing "quickies" was not used in these experiments because artery curing is a method that has not yet been adapted to the farm curing of pork.

Whenever possible, both the right and left hams from the same hog were used, and the measurement for thickness of each was taken through the cushion directly behind the aitch bone.

---

* Authorized for publication, March 10, 1942.

† Professor of Animal Husbandry and Assistant Professor of Agricultural and Biological Chemistry, respectively.

Each ham was sampled by removing a slice 1 inch thick directly back of the aitch bone at the same location that the measurement was taken. A piece 3 inches wide was removed from the center of this cut and divided into five equal horizontal sections for salt analysis, fig. 1. The sample from the outside was designated as subsample 1 and the sample from the aitch bone side or inside as subsample 5. The amount of salt present in each subsample was calculated from the chlorine content, determined by the wet-digestion method used by Davis, supplemented in many cases by readings made on a Meg Salinity Tester.

It might not be amiss to explain why all these tests have been conducted with the 75° and 85° pickles which are considered strong pickles by the pork packing industry. The conditions for handling and curing pork, from the standpoints of bleed, rapidity of dressing, chilling, and controlled refrigeration and cellar temperatures permit the use of the weaker curing pickles in packing plants. The conditions that exist on many farms require stronger pickles for safe curing and even these are not always effective because of certain factors, such as improper bleeding, unclean or contaminated curing vessels, the use of low grade salt, and high curing cellar temperatures.

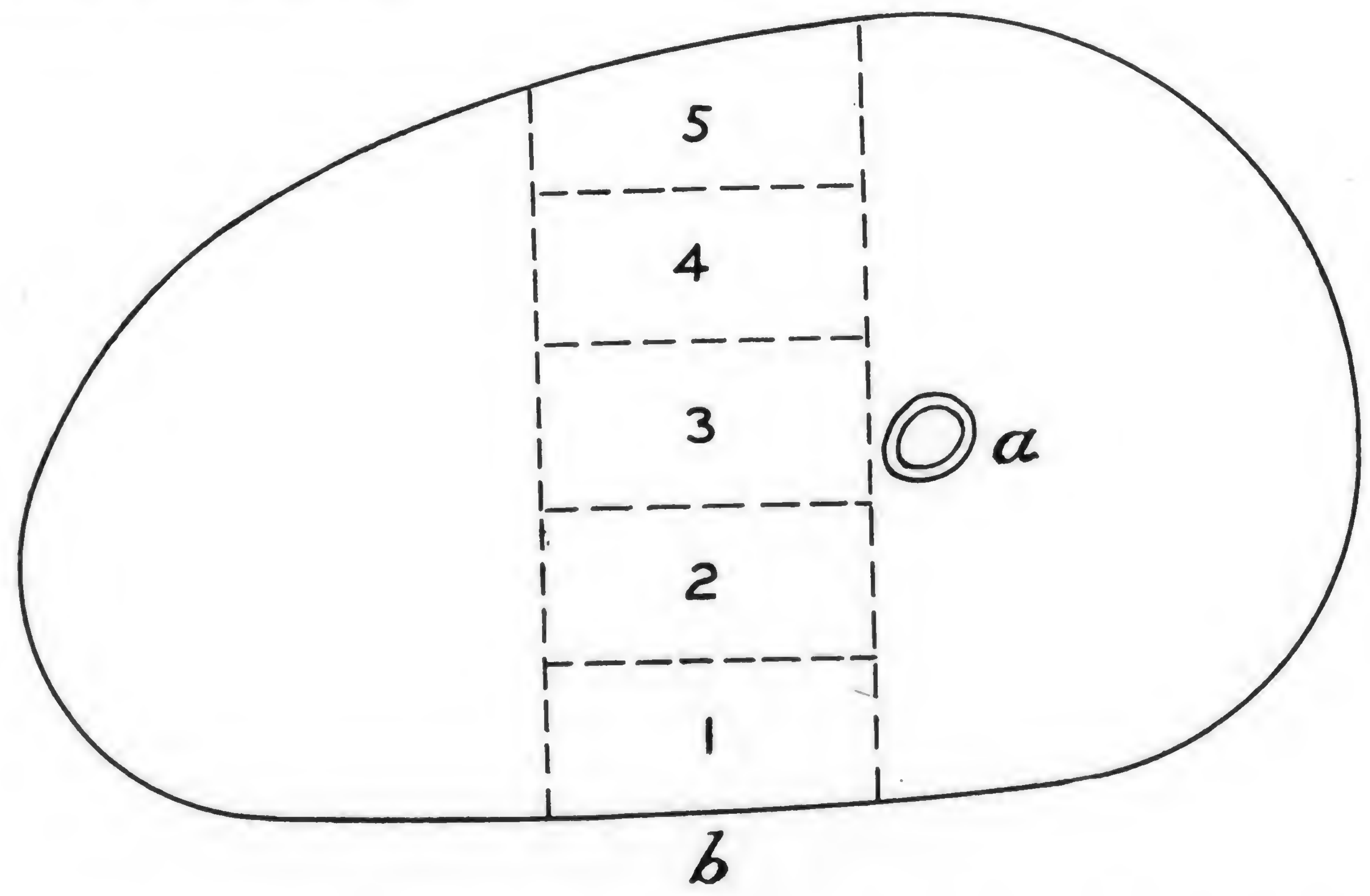


Fig. 1.—The progress of the distribution of salt in ham is indicated by the analysis of test pieces taken through the thickest portion as indicated in this diagram.

CURING SHARP FROZEN HAMS

The establishment of freezer locker plants throughout the United States has supplied the farmer with a means of preservation hitherto unavailable. The advisability of using sharp frozen and locker stored hams for curing purposes was one of the problems presented.

To determine the rate of salt penetration and the palatability and keeping qualities of hams which had been sharp frozen, a test was made on 64 hams which had been frozen at -10° Fahrenheit and held at 0° F for from 1 to 8 weeks. These were divided into 4 groups of 16 hams each and received the following treatment:

Group I; thawed before curing in 85° pickle.—This group was divided into four groups of four hams each which were cured in an 85° pickle for periods of 6, 7, 8, and 9 days, respectively, for each inch of thickness measured through the cushion of the ham.

Group II; placed in 85° pickle without thawing.—This group was divided as in Group I but the sub-groups were not thawed before being placed in an 85° pickle and cured respectively for 6, 7, 8, and 9 days per inch of thickness.

Group III; thawed and placed in 75° pickle.—This group was divided and cured as in Group I.

Group IV; placed in 75° pickle without thawing.—This group was divided and cured as in Group II.

Table 1.—Percentages of salt in different groups of hams, thawed and unthawed, after curing for various lengths of time in 85° pickle.

DAYS CURED PER INCH THICKNESS	SAMPLE NUMBERS; THAWED HAMS					SAMPLE NUMBERS; HAMS LEFT TO THAW IN PICKLE				
	1	2	3	4	5	1	2	3	4	5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	7.23	10.88	9.96	16.83	20.12	5.67	9.15	7.27	14.55	20.7
7	7.76	11.52	9.81	17.36	21.05	5.98	10.22	8.40	15.14	21.4
8	7.52	15.03	13.05	19.29	21.61	6.02	12.77	10.76	16.55	20.9
9	9.75	15.26	13.90	20.01	21.75	8.02	13.62	12.64	17.83	21.2

The hams that were thawed before being placed in cure did not absorb salt any more rapidly than the hams that were allowed to thaw in the pickle when the thawing period was taken into account. The cooking test proved the 8- and 9-days-per-inch cured hams to be slightly salty. A subsequent test of similarly cured hams showed that a cure of 5 days per inch gave a mild cured ham. A cure of 6 days per inch of unthawed ham in 85° pickle produced a very palatable product.

Table 2.—Percentages of salt in different groups of hams, thawed and unthawed, after curing for various lengths of time in 75° pickle.

Days Cured per Inch Thickness	Sample Numbers; Thawed Hams 1	2	3	4	5	Sample Numbers; Hams Left to Thaw in Pickle 1	2	3	4	5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	2.30	4.49	4.43	8.97	15.64	2.07	4.38	4.31	7.99	12.26
7	3.72	9.19	8.66	12.45	17.75	3.26	8.16	7.50	11.95	13.73
8	4.12	10.23	9.95	13.66	18.29	3.82	9.33	8.24	12.87	14.12
9	4.79	12.50	11.35	17.52	20.40	4.36	10.22	8.89	15.65	16.50

Analysis of a well flavored ham of good keeping quality shows the following salt percentages: 5—12—12—14—14. See figure 1 for location and order of samples. This even distribution of salt is brought about by ageing. The hams cured 8 days per inch in the 75° pickle were the most desirable.

The freezing of the hams hastened the rate of cure as established and reported in Bulletin 355 by 3 days per inch of thickness, or about ½ day per pound.

Table 3.—A comparison of rate of cure of fresh and frozen hams.

	Fresh	Frozen
	days per inch	*days per inch*
75° pickle	11	8
85° pickle	9	6

Sample hams from each group were stored in a room averaging 80° F for the three summer months and, except for the development of different degrees of rancidity in the fat, all held well and had excellent flavor. The color was uneven, which was characteristic of all the frozen hams.

PUMPING AND FREEZING

A group of 8 hams were pumped with 8 per cent of their weight of 85° pickle and then placed in a freezer locker at 0° F for 2 weeks. At the end of this period they were placed in an 85° pickle without being thawed. The temperature of the curing room was 38° F.

Table 4.—Average salt percentages of 8 pumped hams that were frozen and placed in 85° pickle without being thawed.

Days Cured per Inch Thickness	Sample Numbers 1	2	3	4	5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
5	6.81	13.28	11.47	17.78	18.79

The hams used in this test averaged 11 pounds in weight and measured 4½ inches in thickness. The length of the curing period was 22 days or 5 days per inch and was figured on the actual time that they were in the pickle.

The object of pumping the hams and then freezing them was to determine the effect on the firmness of the finished product, also the effect on rate of cure and salt equalization.

The hams were firm but not any more tender than others receiving different treatment. The process lowered the period of cure (time actually in the pickle) and gave a salinity similar to the frozen hams that were cured 8 days per inch.

CURING PUMPED HAMS

The strength of the pump pickle used in these experiments was the same as the cover pickle. A standard salimeter was used to check the strengths of the pickles made by dissolving the 8—3—3 formula in 4 gallons of water (85° pickle) and 5 gallons of water (75° pickle). The curing room temperature was 38° F.

Six separate groups of hams were pumped with various percentages of pickle of either 75° or 85° strength and cured respectively, for 5, 6, 7, and 9 days per inch of thickness.

Group I.—These hams were pumped with 8 per cent of their weight of 85° pickle and then rubbed with ½ ounce of 8—3—3 curing mixture per pound of ham. Two rubbings were required and the hams were shelf cured at the rate of 5 days per inch of thickness.

Group II.—These hams were pumped with 8 per cent of their weight of 85° pickle and were then placed in a cover pickle of the same strength for 5 days per inch of thickness.

Group III.—Hams in this group were treated the same as Group II except they remained in cure 7 days per inch of thickness.

Group IV.—These hams received only 3 per cent of their weight of 85° pickle and were placed in a cover pickle of the same strength for a period of 9 days per inch of thickness.

Group V.—These hams received 5 per cent of their weight of 85° pickle and were placed in a cover pickle of the same strength for a period of 6 days per inch of thickness.

Group VI.—These hams were pumped with 10 per cent of their weight of 75° pickle and placed in a cover pickle of the same strength for a period of 7 days per inch of thickness.

All hams in the six groups were soaked for 2 hours in tap water and smoked at a temperature ranging from 110 to 125° F until they had the desired amber shade.

One week later samples were taken and analyzed and cooking tests were made on each ham.

Table 5.—Salt percentages of different groups of hams pumped and cured by varying methods for differing periods of time.

GROUP	DAYS PER INCH IN CURE	SAMPLE NUMBER 1	2	3	4	5	FLAVOR	CURING PERIOD (days per lb.)
I	5	2.62	7.04	8.26	12.63	13.35	Mild	2
II	5	4.24	12.65	11.05	15.37	15.87	Ideal	2
III	7	4.25	15.07	18.50	15.49	13.74	High	2
IV	9	6.22	14.07	13.04	15.86	20.12	High	2½
V	6	5.55	10.80	9.13	10.71	12.92	Mild	2¼
VI	7	4.43	10.48	8.77	13.76	15.39	Ideal	2½

The color of these hams was more uniform than was the case of the frozen lot. The check hams were held from 30 to 90 days and were sound when used.

The curing periods of these groups when stated in days per pound show that Group II, pumped with 8 per cent of their weight of 85° pickle and cured 2 days per pound in 85° pickle, and Group VI, pumped with 10 per cent of their weight of 75° pickle and cured 2½ days per pound in 75° pickle, were well cured in a fairly short curing period. All of the other processes successfully cured hams.

CURING UNCHILLED HAMS

The ensuing report may sound rather fantastic, but results speak for themselves. The hams were taken from two hogs immediately after dressing. The inside temperatures of the hams registered 101°F. They were skinned and given a thorough rubbing with the 8—3—3 curing formula and some of the cure was punched into the joint close to the aitch bone. The hams were placed on a table in a room where temperatures never went below 60°F. They were given 2 more rubbings at 4-day intervals, or a total of 1¼ ounces of cure per pound of ham. They remained in the warm room for a period of 7 days per inch of thickness or 35 days (5-inch hams-14 pounds) or 2½ days per pound.

At the end of 35 days they were soaked in tap water for 2 hours. They were then allowed to dry before being smoked. All four hams were sound and had excellent flavor when used, but had lost considerable weight. This would appear to disprove the necessity of chilling, but it must be recalled that salt was immediately introduced to the ham joint. It further proves the value of the dry sugar cure method for farm conditions.

SUMMARY

Sharp frozen hams or hams frozen in freezer lockers at 0°F to -10°F and held in storage for 1 to 8 weeks were cured in 3 days less time per inch of thickness or ½ day less per pound of ham than unfrozen hams.

It was not found necessary to thaw hams before placing them in cure. Allowing frozen hams to thaw in pickle kept down the temperature of the pickle for a longer period, which was an advantage where there was no means of controlling the curing cellar temperature.

A curing period of 6 days per inch of thickness when frozen hams were placed in an 85° pickle, and 5 days per inch if previously thawed and covered with a 85° pickle, gave the most desirable salt content. This was the equivalent of 2 days per pound of ham.

Hams that were locker frozen and then cured were sound and very palatable after being held for three months in a summer storage (farm conditions) at 80°F.

Forcing the pickle into the center of the ham by means of a ham pump reduced the curing period from 3 to 4 days per inch of thickness, or about ¾ to 1 day per pound of ham when they were pumped with 8 per cent of their own weight of pickle.

Hams pumped with 8 per cent of their weight of 85° pickle and then sharp frozen for 2 weeks were cured in a cover pickle of the same strength in 5 days per inch of ham thickness, or 1 day per pound of ham less than fresh hams.

When hams were pumped with 10 per cent of their weight of 75° pickle and cured in a cover pickle of the same strength, the optimum curing period proved to be 7 days per inch of ham thickness or 2½ days per pound of ham. This is 4 days less per inch of ham than is necessary for unpumped hams cured in 75° pickle.

Unchilled hams were successfully dry sugar cured at a room temperature of 65°F in 35 days or 7 days per inch of ham thickness (2½ days per pound) using 1¼ ounce of the 8—3—3 curing formula per pound of ham.

TENDERIZED HAM

Although these experiments did not include work on artery curing, a brief description of the packers' methods of quick curing and tenderizing hams is in order. Artery curing consists of forcing a 60° pickle into the femoral artery on the inside and butt end of the ham by means of a small needle attached to a hose and connected to a pump that exerts a pressure of 40 pounds. The ham is placed on a scale, the needle is inserted into the artery which is held tightly over the needle, and 10 per cent of the ham's weight in pickle is forced into the arterial circulatory system. This bathes all the cell tissue with the pickle, diluting any blood that remains in the arteries, and effects a cure in 12 to 15 days regardless of the size of the ham. A 10-pound ham would receive 1 pound, and a 15-pound ham 1½ pounds of pickle (10 per cent by weight); thus the quantity necessary to affect an immediate cure is already placed in that section and need not seep there. The pink color that is characteristic of cured ham is obtained by including 1 pound of sodium nitrite with every 9 pounds of sodium nitrate. The use of nitrate alone gives the enzyme action in the ham insufficient time to convert the nitrate into nitrite, a necessary process for color fixation.

Several types of tenderized hams under various trade names are on the market. These consist of "ready to slice" ham which requires further cooking in the home, and "ready to eat" ham which can be consumed with or without further preparation. The hams are first soaked in tap water to remove excess outside salt and are then placed in a smoke house. It takes about 24 hours to smoke and produce tenderized hams, and the process is usually accomplished in three stages. During the first eight hours, or the drying stage, gas burners, or steam, if coils are used, heat the house to 125°F. All drafts are open to carry off excess moisture and there is no smoking during this period. During the next 8-hour stage, the drafts are closed about half way, the temperature is raised to 135°F, and smoke is generated. The smoking continues throughout the third stage with all drafts closed, and the temperature is raised to 165° until the inside temperature of the ham reaches 142°F when it is removed. The resulting product constitutes the "ready to slice" ham.

To produce "ready to eat" ham, the inside temperature of the hams must reach 155°F and be held at that temperature or above for another 2 hours. Trichinae which is found in a small percentage of our pork supply is killed by a temperature of 140°F and thus "ready to eat" ham is entirely safe in this respect. This method of tenderizing hams can be used with only a small loss in weight.

250

NUTRITIONAL DEFICIENCIES OF A CONCENTRATE MIXTURE COMPOSED OF CORN, TANKAGE, SOYBEAN OILMEAL, AND ALFALFA MEAL FOR GROWING PIGS

BY

T. B. KEITH, R. C. MILLER, W. T. S. THORP,
AND M. A. McCARTY

Reprinted from
JOURNAL OF ANIMAL SCIENCE
Vol. 1, No. 2, May, 1942

NUTRITIONAL DEFICIENCIES OF A CONCENTRATE MIXTURE COMPOSED OF CORN, TANKAGE, SOYBEAN OILMEAL, AND ALFALFA MEAL FOR GROWING PIGS[1]

T. B. Keith, R. C. Miller, W. T. S. Thorp, and M. A. McCarty
Pennsylvania Agricultural Experiment Station

IN a series of studies dealing with the protein requirement of the growing pig, conducted during the past five years, a number of abnormal conditions (such as incoordination and lameness, growth failure, vomiting, diarrhea, inactivity, anorexia, and a mild dermatitis) have been encountered. This report deals with some observations on certain numbers of individual pigs which were fed in an effort to obtain further information concerning these disorders.

Observations on Stiffness

Among the disorders was a type of stiffness and lameness which was encountered with pigs that had gained weight unusually fast. These pigs were started on experiment at between 70 and 100 pounds live weight and were fed a mixture comprised of corn, tankage, soybean oilmeal, sun-cured ground alfalfa hay, and salt. All rations contained 4 per cent of the alfalfa meal. The quantity of the other feeds was varied to furnish rations of different total protein contents as desired. The percentage of tankage was varied from 4 to 25 per cent of the ration. Low protein bone meal tankage and 55 per cent protein digester tankage were used.

Stiffness occurred at about the time the pigs reached a body weight of 150 pounds. The condition was at first characterized by a slight halting of the gait, which slowly progressed in severity until it was only with difficulty that the pigs could rise to their feet. Their behavior indicated that the condition was painful. Most of the pigs would lie down continually and if forced to get up would not remain standing. Some of the animals would sit on their buttocks and practically all of the severely afflicted would walk on their knees. None appeared to be dizzy or showed any indication of a lack of muscular control. They continued to gain and had normal appetities and were otherwise thrifty and healthy. Their skins were clean and oily, the hair was sleek, and the eyes were clear and appeared normal in every respect with the vision unimpaired.

Thirty out of a total of 60 pigs were affected with stiffness. During the period of the experiments, 9 of the unaffected pigs did not attain the weight of 150 pounds. Hence, it may be said that 30 out of 51 pigs which attained a weight of 150 pounds became stiff.

In outward appearance the condition of the afflicted individuals closely resembled rickets. However, the condition was not alleviated or affected in any way by the administration of cod-liver oil or irradiated yeast. Subsequent analysis of the ulnae and radii of 18 affected pigs showed that the mineral content of these bones was normal, the average value for bone ash being 58.92 per cent on the dry, fat-free basis. Normal values were also obtained for the inorganic phosphorus content of the blood. However, there were certain constantly occurring changes observed in the leg bones which indicated abnormal development. Upon gross examination the distal extremity of the radius and of the ulna showed enlargement. Roentgenographs of this area of these bones indicate that there was an increased activity of the tissues at the oesteochondral junction, this area being definitely enlarged, but there was no apparent irregularity of the zone of ossification and no other changes present other than hypercalcification, thus differentiating the condition from rickets. The occurrence of the stiffness was first noticed during the feeding of rations which had relatively high mineral contents, ranging from 6 to 9 per cent. On analysis, these rations were found to have low manganese contents ranging from .0011 to .0014 per cent.

Manganese sulphate was then added to the swine rations in amounts to supply 50–60 parts per million of manganese. As a result, 15 pigs which received the manganese supplemented rations showed no signs of stiffness. Fifty per cent of litter and pen mates of these same pigs fed the unsupplemented rations became stiff. However, the addition of manganese to the ration was ineffective in curing stiffness after it had developed.

Observations on Further Deficiencies of the Basal Ration

In a later experiment, unusual cases of vomiting, diarrhea, anorexia, growth failure, and ataxia were observed in a group of 10 pigs fed the basal ration, presented in table I, during the winter of 1939. Five of these pigs were fed two-tenths pound of manganese sulphate with each 100 pounds of the basal ration, the rations of the remaining pigs having manganese contents of .0011 to .0014 per cent. These pigs were smaller and younger at the beginning of the feeding test than those used in the previous studies, their initial weights ranging from 26 to 40 pounds. The rate of gain of these pigs was very slow as compared to those showing the stiffness. At the end of 70 and 84 days, respectively, two pigs had died. One of these pigs had lost 5 pounds and the other had lost one pound. Both individuals had had severe spasms of vomiting, nausea, and diarrhea, they huddled together, ate very little, a mild dermatitis developed and small lesions were present on their face and legs. These pigs were properly injected with nicotinic acid and were fed riboflavin and pyridoxine with no beneficial results.

---

[1] Authorized for publication on November 25, 1941, as paper No. 1064 in the Journal Series of the Pennsylvania Agricultural Experiment Station, State College, Pennsylvania.

Four of these pigs were fed the ration for a period of 147 days. Abnormal conditions were evident, but not serious. Their gains were subnormal, averaging about .5 pound per day. Cod-liver oil and nicotinic acid given to two of these pigs failed to increase the rate of gain or to improve their condition.

Another pig was fed 100 grams daily of vacuum dried pork liver meal after having a loss of body weight of 5 pounds for the first 98 days of feeding. This pig was weak and had all of the afflictions of the two pigs that died. After 63 days of feeding the liver supplement, the pig had gained 53 pounds and appeared normal in every respect.

The above facts indicate that the basal ration was deficient in one or more nutritional factors important with respect to growth, normal digestion, muscular control, and normal functioning of the skin. A study was then outlined to give further information concerning the observed deficiencies.

The ration used contained the same ingredients in similar proportions as those used in the manganese studies, table 1.

Forty pigs were selected soon after weaning at ages ranging from 48 to 60 days and weights ranging from 18 to 30 pounds.

TABLE 1.—CONCENTRATES USED IN THE BASAL MIXTURES[1]

Concentrates	Basal	Basal ration with a portion of tankage replaced by soybean oilmeal
Yellow corn (No. 2)	60.5[2]	60.5
Low protein tankage	35.0	25.0
Soybean oilmeal (expeller)	—	10.0
Ground sun-cured alfalfa	4.0	4.0
Salt	.5	.5

[1] All pigs were fed in individual stalls on concrete floors with access to concrete outside runways.

[2] Parts per 100 lbs.

The outline of the treatments was as follows:

(1) Basal and 100 grams of yeast daily. At the end of 56 days, 40 gamma of beta carotene were fed each day.

(2) Basal and 40 gamma of beta carotene daily. At the end of 56 days, 100 grams of yeast were fed each day.

(3) Basal and a vitamin mixture of nicotinic acid 75 mgm., thiamin chloride 5 mgm., riboflavin 2 mgm., calcium pantothenate 10 mgm., and pyridoxine 0.8 mgm. At the end of 56 days, 40 gamma of beta carotene were fed each day.

(4) Basal. At the end of 56 days, an alcoholic extract of whole vacuum dried beef liver was fed daily.

(5) Basal. At the end of 56 days, 100 grams of whole vacuum dried beef liver were fed each day.

(6) Basal and 100 grams of whole vacuum dried beef liver daily.

(7) Basal with 10 parts of the tankage replaced by 10 parts soybean oilmeal. At the end of 56 days, 100 grams of brewers' yeast and 40 gamma of beta carotene were fed each day.

(8) Basal with 10 parts of the tankage replaced by 10 parts of soybean oilmeal and 100 grams of brewers' yeast daily.

(9) Basal with 10 parts of the tankage replaced by 10 parts of soybean oilmeal and 40 gamma of beta carotene daily.

At the end of 56 days of feeding, all the pigs fed the basal ration showed the following symptoms: emaciation, weakness, vomiting, anorexia, diarrhea, and a mild dermatitis.

The feeding of 100 grams of brewers' yeast daily with the basal ration did not correct the deficiencies of the ration, treatment 1. Three of the 5 pigs died during the course of the feeding period; the other 2 failed to gain and exhibited the same deficiency symptoms as those fed the basal ration. After 56 days of feeding, 40 gamma of beta carotene were fed each day to these 2 pigs, with no improvement in their condition.

The feeding of 40 gamma of beta carotene daily during the first 56 days and 40 gamma of beta carotene and 100 grams of yeast with the basal ration during the remainder of the period, did not fulfill the nutritive requirements of these pigs, treatment 2.

The addition of the mixture of the B vitamins to the basal mixture did not supply the necessary essential nutrients, treatment 3. Two of the 5 pigs died; the other 3 were definitely emaciated with characteristic symptoms observed in the other pigs fed the basal ration. The feeding of 40 gamma of beta carotene each day to each of these 3 pigs, beginning at the end of 56 days, did not improve their condition.

A limited response was noted with each of the two pigs that were fed the alcoholic extract of the vacuum dried beef liver, after 56 days on the basal ration, treatment 4. The recovery of these 2 pigs was not as rapid or as complete as that of the pigs fed whole vacuum dried beef liver. Complete recovery and normal growth ensued with the 3 pigs fed the 100 grams of whole vacuum dried beef liver each day, after 56 days on the basal ration, treatment 5.

Of the 5 pigs fed the basal ration and 100 grams of the liver each day, treatment 6, three pigs died during the first 30 days of feeding. Internal hemorrhages caused the death of these pigs. The cause of the internal hemorrhages has not been determined. The other 2 pigs made an average daily gain of .94 pound. At the end of 98 days both pigs were showing an incoordination, one pig was passing bloody urine, and a mild dermatitis was present on the ears, legs and tails of both pigs. The ataxia did not develop into a severe stage, yet there were no changes in the gaits of these animals after a subsequent 5 weeks on alfalfa pasture.

Of the 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal, 2 died before the end of the feeding period, treatment 7. These 2 pigs exhibited the same symptoms as those fed the basal ration. The other 3 pigs gained rather slowly. The daily allowance of 100 grams of brewers' yeast and 40 gamma of beta carotene to the 3 pigs, beginning at the end of 56 days, brought about an increase in the rate of gain during the last 49 days.

The 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal and 100 grams of brewers' yeast daily per pig, made an average daily gain of .60 pound with individual gains ranging from .42 to .97 pound, treatment 8. Two individuals had a mild dermatitis, and one of these had ataxia.

The 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal and 40 gamma of beta carotene daily, made an average daily gain of .71 pound with a range of .58 to .88 pounds for individual pigs. One pig had a moderate dermatitis, and ataxia developed at about 105 days. After feeding on alfalfa pasture for three weeks, the ataxia became more pronounced, and within a short time the animal was unable to walk, treatment 9.

Histological Studies

Histopathological studies were made on the liver, kidney, skin, and, in several cases, on the spinal cord and sciatic nerves. The skin lesions have been described as a mild dermatitis; however, it resembles more closely an epidermidosis since the lesions were confined principally to the epidermal layers. In only two cases examined microscopically did the dermatitis extend below or through the Malpighian or germinal layer. No significant pathology can be reported for the liver and kidney at this time.

Fresh sections of the spinal cord and the sciatic nerve from pigs, on treatment 9, were fixed and stained according to Marchi's technique for degeneration of the myelin sheath. Microscopic examination of this material revealed a moderate degeneration of the myelinated tracts with some fragmentation of the demyelineated nerve fibers. This was observed to a variable degree in both the sciatic nerves, the dorsal columns of the spinal cord and dorsal nerve roots. These myelin changes were not as extensive as those

reported by Wintrobe, Miller, and Lisco (1940) and by Ellis and Madsen (1941).

Summary

The addition of manganese to rations which were high in ash and low in manganese content was found to be of benefit in preventing stiffness with pigs of initial weights of 80 to 100 pounds which were gaining weight rapidly. No other deficiencies were evident with normal healthy pigs of these initial weights.

For young pigs with initial weights ranging between 20 and 35 pounds at the beginning of the experiment, rations comprised of yellow corn, tankage, alfalfa, and salt were deficient in growth factors. The factors lacking are apparently supplied by liver and a combination of soybean oilmeal, yeast, and carotene. These factors are not supplied by yeast alone or pure B vitamins.

Nutritional factors required to prevent a mild dermatitis and ataxia were not fully supplied by any of these supplements.

Literature Cited

Ellis, N. R. and L. L. Madsen. 1941. Relation of Diet of Swine to Development of Locomotor Incoordination Resulting from Nerve Degeneration. Jour. Agr. Res., 62: 303–316.

Wintrobe, Maxwell M., Joseph L. Miller, Jr., and Herman Lisco. 1940. The Relation of Diet to the Occurrence of Ataxia and Degeneration in the Nervous System of Pigs. Bul. of Johns Hopkins Hosp. 377–405.

Reprinted from The Journal of Nutrition
Vol. 23, No. 5, May, 1942

EFFECT OF MANGANESE ON CALCIFICATION IN THE GROWING RAT

CHARLOTTE CHORNOCK, N. B. GUERRANT AND R. ADAMS DUTCHER
Department of Agricultural and Biological Chemistry, Pennsylvania State College

TWO FIGURES

(Received for publication November 25, 1941)

Numerous experiments have been reported concerning the question of whether cations other than calcium can be deposited in the bones. Some of the results are of interest purely as experimental phenomena, indicating that the calcification mechanism is not absolutely specific for calcium salts; some, however, have shown the fate and to a large extent have guided the therapy of certain toxic metals in the body. Examples of metals used in such studies are lead, strontium, beryllium, radium, bismuth, and manganese.

Manganese, like beryllium, aluminum, and iron, has proved rachitogenic when fed in large amounts (Blumberg, Shelling and Jackson, '38). Since the major part of this element is excreted in the feces (Skinner and Peterson, '30), the harmful action is probably caused, not by the toxicity of manganese itself, but by its effect on phosphorus metabolism. Because appreciable amounts of manganese itself are absorbed and found in various tissues, including bone, there is also the possibility that it may have a direct influence on the calcifying mechanism.

Recent work has indicated that traces of manganese (25 to 50 p.p.m.) in the diet of chicks prevent perosis (Gallup and Norris, '39 a). A requirement as definite as that suggested for the chick has yet to be demonstrated in the rat, although manganese appears to have an important role in the production and rearing of normal young of both rats and chickens (Gallup and Norris, '39 b; Orent and McCollum, '31; Barnes, Sperling and Maynard, '41).

The ingestion of certain acidic organic compounds has been reported to influence the cure of rickets by improving mineral absorption (Hathaway and Meyer, '39; Shohl, '37). Since manganese complexes with organic acids were observed by Delsal ('38), the effect of citrate on the mineral elimination in the feces was believed to constitute an important phase of any series of manganese metabolic studies.

The present investigation deals with three problems in manganese metabolism: (1) the effect of large manganese intakes on the fecal output of calcium and phosphorus and on the amount and location of the stored manganese; (2) the effect of phosphate, vitamin D, and citrate on both mineral excretion in the feces and the cure of rickets in the presence of manganese; (3) the probable role of very small manganese intakes in calcification.

EXPERIMENTAL

The experimental work with manganese in the diet was divided into two parts, the first dealing with high manganese intake and the second with small amounts of manganese.

High manganese levels

Young rats weighing between 45 and 50 gm. were depleted of their body stores of vitamin D by feeding some groups the Steenbock and Black ration no. 2965, and other groups the same ration with the calcium carbonate molecularly supplemented or replaced by manganous carbonate. The rachitogenic diets supplemented with manganese are designated as 1/5 S, 2/5 S, 3/5 S, and 5/5 S; while the rations in which the calcium was replaced by manganese are designated as 1/5 R, 2/5 R, 3/5 R, 5/5 R, 2/5 Rp, and 5/5 Ra. Diet 2/5 Rp had the phosphorus content increased by the addition of 1% potassium dihydrogen phosphate; in diet 5/5 Ra, 0.5% of the calcium carbonate was left in the diet (similar to diet C of Blumberg, Shelling and Jackson, '38). The calcium, phosphorus, and manganese contents of these diets on a dry basis were as follows:

DIET	Ca %	P %	Mn %	DIET	Ca %	P %	Mn %
Steenbock	1.48	0.36	0.002	1/5 R	1.31	0.36	0.42
1/5 S	1.50	0.36	0.312	2/5 R	1.12	0.36	0.68
2/5 S	1.52	0.36	0.66	2/5 Rp	1.16	0.80	0.66
3/5 S	1.55	0.32	1.12	3/5 R	0.93	0.33	1.02
5/5 S	1.40	0.32	1.73	5/5 R	0.08	0.40	1.58
				5/5 Ra	0.84	0.51	1.22

All animals were placed in a darkened room in individual metal cages provided with raised screen floors. They were given ample rachitogenic diet and distilled water. A record was kept of food intake and change in body weight.

After a 21-day depletion period, half of the animals on each diet were killed by anesthesia in order to obtain blood and bones for analyses. The remaining animals were given (1) 5 units of vitamin D (U. S. P. XI reference cod liver oil), (2) 5 units of vitamin D with potassium citrate in the diet (0.4 mols per 100 gm.), or (3) 100 units of vitamin D. At the end of a 7-day curative period, these animals also were killed.

The blood samples obtained by cardiac puncture were pooled for each group of animals. Serum inorganic phosphorus was determined by the method of Youngberg and Youngberg ('30) and calcium by the Clark-Collip modification of the Tisdall method ('25). Livers were pooled for each group of animals. These were dried at 100° C., ground in a glass mortar, and ashed. The ash was analyzed for manganese by the periodate method of Willard and Greathouse ('17).

The feces were collected from individual animals during the 21-day depletion period and during the 7-day curative period. The dried fecal pellets were weighed and pulverized. Duplicate samples were ashed in platinum crucibles. The ash was dissolved in 5 ml. of nitric acid (1:4) by the addition of 1 to 2 ml. of hydrogen peroxide (3%) and the acid solution washed through a quantitative filter paper into a 100 ml. volumetric flask and diluted to volume with distilled water. Aliquots of this solution were used for analysis.

Phosphorus was determined by the volumetric method with ammonium molybdate precipitation (Hillebrand and Lundell, '29, p. 567) and calcium by permanganate titration of the oxalate in the presence of acetic acid (Ibid, p. 501). Manganese was determined by the periodate method, colorimetric measurement being made with an electrophotometer using a green filter with maximum absorption of 525 mμ.

Low manganese levels

The effect of small amounts of manganese on calcification was studied in the following experiments.

1. The Steenbock diet was supplemented with 0.005% and 0.01% manganese added as the carbonate.
2. The Steenbock diet was supplemented with tri-weekly subcutaneous injections of manganese lactate solution. The injections were made at levels of 2.5 mg. and 5.0 mg. per week; these amounts are equivalent to those in 50 gm. of diets containing 0.005 and 0.01% of manganese respectively. A level of 50 mg. per week was fatal 14 hours after the first injection. This high level was tolerated by adult rats, but their weight remained stationary, and after 2 weeks they were in very poor condition.
3. A non-rachitogenic, manganese-free, but otherwise adequate diet was used as a control. It consisted of whole milk powder [1] with supplements of 70 mg. of iron and 15 mg. of copper per week. No manganese could be detected in the ash of a 20-gm. sample of this milk powder by the method used. Manganese was added to the diet as a manganous sulfate solution containing 2.5 mg. manganese per milliliter. This was fed at levels of 1.25 and 2.5 mg. per week, representing 0.0025 and 0.005% dietary levels at a food intake of 50 gm. per week. The solutions of ferric chloride, copper sulfate, and manganous sulfate were mixed with 30 gm. of milk powder and dried. After the animals had consumed this mixture, they were allowed to eat the whole milk powder freely the remainder of the week. The groups were designated as MP, MP .0025, and MP .005.

---

[1] Klim.

The groups of young rats receiving these diets and supplements were subjected to the same procedures for the depletion and curative periods as described for the animals on high manganese levels with the exception of the rats in the milk powder groups. These animals received no vitamin D and were kept on the diet for 5 weeks.

RESULTS AND DISCUSSION

The average values for the results obtained from each group of animals are given in tables 1 and 2 and figures 1 and 2.

The growth response (table 1) indicated clearly the retarding effect of high manganese intake on growth in rats receiving the Steenbock diet. Considering the effect of manganese supplements with a high calcium-low phosphorus diet, it was evident that the growth of the animals decreased as the amount of manganese in the diet was increased (fig. 1). With equal amounts of calcium and manganese, the animals lost weight during the entire experimental period, losing about 30% of their initial weight in 4 weeks.

The condition of the animals may be explained by the abnormal calcium-phosphorus metabolism induced by the Steenbock diet itself and intensified by the presence of large amounts of manganese. The calcium excretion was increased with increased manganese intake (table 1). The phosphorus excretion was not only increased, but at the higher manganese levels, negative phosphorus balances were indicated by the fecal excretion of amounts of phosphorus greatly in excess of the dietary intake.

The replacement of calcium with manganese without increasing the actual mineral content of the diet produced a similar effect on growth and mineral absorption (table 1; fig. 2). Because a partial replacement of the calcium lowered the calcium content toward a more normal calcium-phosphorus ratio, the condition was less severe. The presence of manganese was still sufficient, however, to give a negative phosphorus balance.

TABLE 1

Average food intake, weight change, and mineral balance of the groups of rats receiving high manganese diets.

DIET	NO. OF RATS	FOOD INTAKE (DRY)	MINERAL INTAKE MG./RAT/DAY			VITAMIN D INTAKE	CHANGE IN WEIGHT	SERUM MG./100 ML.		BONE ASH	LINE TEST [2]	PER CENT INTAKE EXCRETED IN FECES			MANGANESE IN	
			Ca	P	Mn			Ca	P			Ca	P	Mn	Bone ash	Liver mg./100 gm. (dry)
							21-day depletion period									
		gm.				*units*	*gm.*			%					%	
Steenbock	80	108	75.5	18.4	0.1		20.9	11.9	3.6	34.5		67.6	76.5	80.0		
1/5 S	24	95	70.5	16.9	14.6		9.3	11.6	4.1	36.5		77.6	83.5	95.9		
2/5 S	42	93	73.0	17.2	31.6		5.9	11.6	3.1	33.8		70.8	102.9	93.4		
3/5 S	25	85	71.3	14.7	51.5		1.6	11.0	3.5	36.9		72.9	125.2	68.9		
5/5 S	8	84	58.8	13.4	72.6		— 7.5		2.3	36.7		76.7	120.1	79.2		
1/5 R	9	93	59.0	16.2	18.8		11.1	13.4	2.6	39.3		69.5	108.6	81.9		
2/5 R	25	93	57.8	15.8	29.9		8.5	11.4	2.6	34.1		56.7	126.5	85.7		
2/5 Rp	4	109	60.2	41.5	34.3		17.3	10.8	3.7	33.3		66.7	61.2	55.6		
3/5 R	9	90	41.8	14.8	46.0		4.1		2.3	37.1		45.4	126.6	76.3		
5/5 R [1]	4	33	2.0	10.0	39.5		—10.0		4.6	44.5		1075.0	113.0	74.4		
5/5 Ra	6	63	24.4	14.8	45.9		— 9.6		2.9	39.8		59.0	85.2	81.2		
							7-day curative period									
Steenbock	24	34	80.0	19.4	0.11	5	3.2	12.3	5.5	35.3	1.5	60.7	68.0	81.8	—	0.83
Steenbock	4	31	65.0	15.8	0.09	100	2.7	13.0	4.7	36.6	2.5	56.8	47.5	77.8		
Steenbock	4	44	90.3	21.9	0.14	5 + K Cit	3.3	10.0	6.1	36.0	3.5	35.4	26.4	78.5		
1/5 S	12	31	66.0	15.8	13.7	5	0.9	12.1	3.7	34.6	—0.3	57.2	74.0	86.1		
2/5 S	12	27	60.8	14.4	26.4	5	1.7	12.6	3.4	33.0	—0.8	59.8	84.0	72.4		
2/5 S	4	25	54.8	12.9	23.7	100	0.3		4.6	29.5	—1.8	72.9	104.7	86.1		
2/5 S	4	27	56.0	18.4	26.4	5 + K Cit	2.3		5.3	31.3	0.7	65.5	57.2	64.4		
3/5 S	8	26	62.0	12.8	44.8	5	— 2.1	12.6	3.2	34.8	—1.0	62.8	115.6	62.3	+	
5/5 S	4	22	43.4	9.9	53.7	5	— 6.0		3.3	31.7	—2.0	71.4	121.2	79.4	+	
1/5 R	5	34	63.0	17.2	20.2	5	3.4	12.2	4.5	33.4	—0.6	48.6	87.2	64.9	+	
2/5 R	12	26	40.4	13.0	24.5	5	— 0.3	12.2	4.1	31.3	—1.5	47.7	102.3	76.3	+	2.81
2/5 R	4	24	40.4	12.9	24.2	100	2.2	12.0	4.2	28.8	—1.3	65.7	89.9	67.7		
2/5 R	4	25	51.8	17.4	22.9	5 + K Cit	3.0	12.0	4.6	32.6	1.5	40.8	45.4	72.9		
2/5 Rp	4	39	63.9	44.0	36.2	5	3.0	11.6	6.0	36.8	2.5	34.3	45.9	65.4		1.71
3/5 R	6	27	37.0	13.2	40.9	5	— 2.8	13.0	3.8	34.5	—2.0	45.0	94.7	69.1	+	5.20
5/5 Ra	6	20	24.4	14.7	45.7	5	— 2.0	12.8	2.9	36.4	0.2	42.1	68.7	64.3	0.007	3.92
												5/5 R (for 14 days)				6.83

[1] Values given for 14 days instead of 21 days.

[2] Where a line test of (—1) indicated mild rickets, (—2) moderate rickets, (—3) severe rickets, and (—4) extreme rickets.

TABLE 2

Average food intake, weight change, and mineral balance of the groups of rats receiving low manganese diets.

DIET	NO. OF RATS	FOOD INTAKE (DRY)	MINERAL INTAKE MG./RAT/DAY			VITAMIN D INTAKE	CHANGE IN WEIGHT	SERUM MG./100 ML.		BONE ASH	LINE TEST [3]	PER CENT INTAKE EXCRETED IN FECES			MANGANESE IN	
			Ca	P	Mn			Ca	P			Ca	P	Mn	Bone ash	Liver mg./100 gm. (dry)
							21-day depletion period									
		gm.				*units*	*gm.*			%					%	
Steenbock	80	108	75.5	18.4	0.10		20.9	11.9	3.6	34.5		67.6	76.5	80.0		
0.005% Mn	32	116	77.0	19.9	0.37		20.8	12.2	3.3	33.4		77.0	79.5	89.2		
0.01% Mn	48	113	74.5	21.6	0.68		19.2	11.8	3.4	37.1		70.6	67.2	102.9		
0.005% Inj. [1]	4	114	86.0	20.8	0.12		22.5					47.7	54.8	266.7		
0.01% Inj. [2]	6	97	45.2	15.8	0.09		12.2					72.7	83.6	455.6		
MP	4	119	62.8	55.8	. . . .		54.1					49.3	30.5	. . . .		
MP .0025	4	111	58.2	52.0	0.18		53.5					42.8	27.0	55.6		
MP .005	4	113	55.0	49.0	0.36		50.0					51.8	30.0	55.5		
							7-day curative period									
Steenbock	24	34	80.0	19.4	0.11	5	3.2	12.3	5.5	35.3	1.5	60.7	68.0	81.8	. . . .	0.83
0.005% Mn	12	38	73.5	18.9	0.35	5	5.4	12.6	5.1	33.5	1.5	62.0	66.7	88.6		0.89
0.01% Mn	16	36	67.7	19.6	0.56	5	4.9	12.0	5.7	36.5	1.5	65.5	63.2	100.0	. . . .	0.99
0.005% Inj. [1]	4	27	54.8	13.3	0.07	5	2.0	13.0	4.0	34.4	—0.1	65.7	90.5	700.0	0.014	1.70
0.01% Inj. [2]	6	31	65.1	15.8	0.09	5	4.0	13.1	3.2	36.1	0.8	55.0	72.2	722.2		2.10
MP	4	56	89.0	79.5	. . . .		21.0	12.2	10.4	56.6	4.0	52.8	27.3	. . . .	. . . .	0.28
MP .0025	4	55	86.8	77.4	0.18		24.0	12.6	10.7	55.8	4.0	54.7	33.1	61.2		0.42
MP .005	4	54	83.5	74.3	0.36		22.0	11.6	11.2	56.0	3.6	50.7	33.6	61.1	. . . .	0.65

[1] Injections averaged 0.36 mg. manganese/rat/day.

[2] Injections averaged 0.71 mg. manganese/rat/day.

[3] Where a line test of (1) indicated just perceptible healing, (2) distinct healing, (3) advanced healing, and (4) normal bone or complete healing.

Addition of phosphate to the diet nullified the action of manganese on phosphorus excretion and improved the appetite and general condition of the animals (diet 2/5 Rp). It is notable, however, that excess of both calcium and phosphorus in this diet allowed a marked increase in manganese retention during the depletion period.

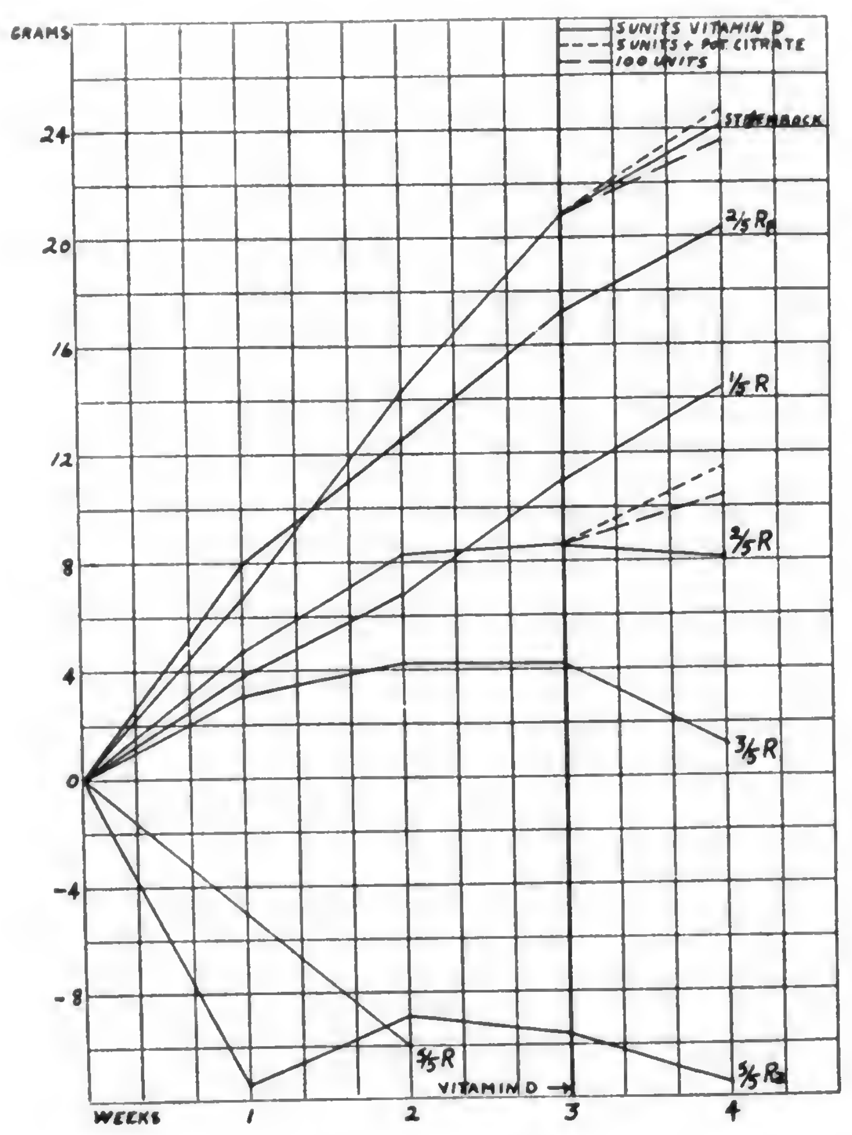


Fig. 1 Growth response in rats in the presence and absence of vitamin D when dietary calcium is supplemented with large amounts of manganese.

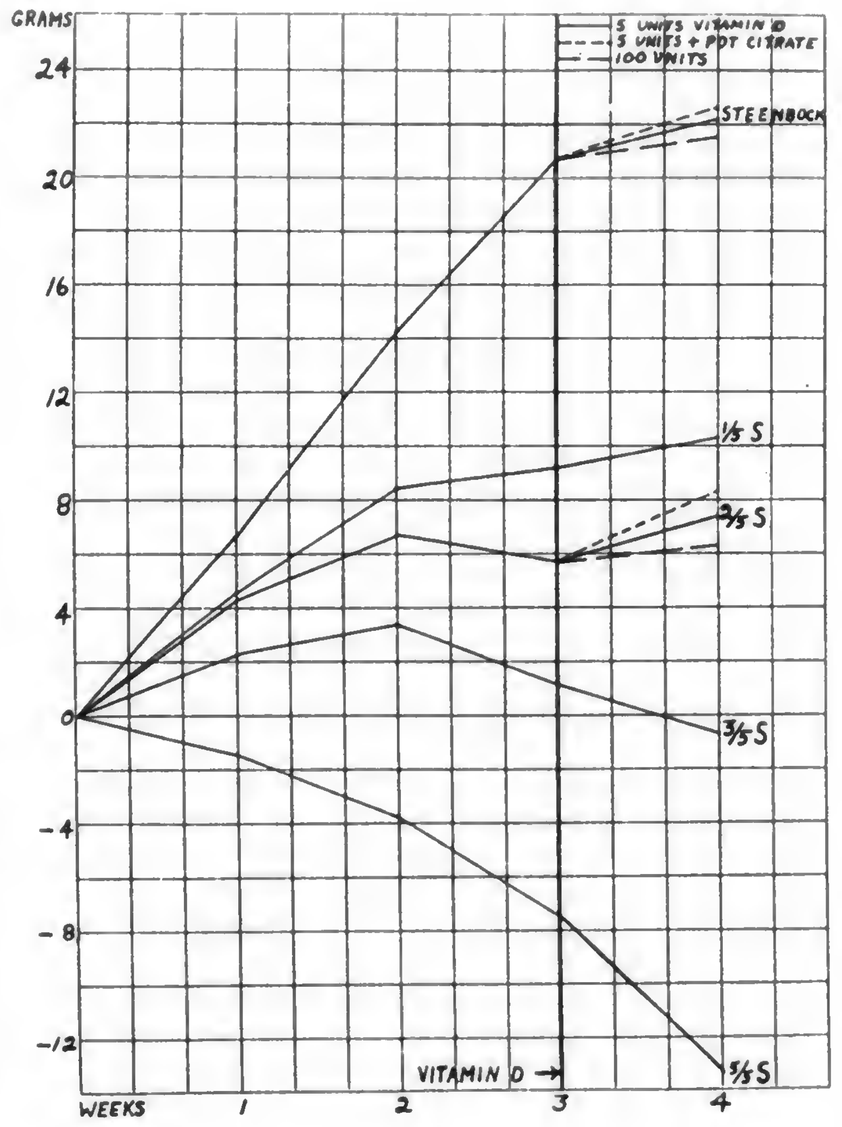


Fig. 2 Growth response in rats in the presence and absence of vitamin D when dietary calcium is partially or entirely replaced by manganese.

The complete replacement of calcium and manganese (diet 5/5 R) resulted in a serious upset in mineral metabolism. Within 14 days some of the animals had died; the others were in very poor condition. The rapid fecal excretion of calcium and phosphorus from the body supply was fatal. Calcium was excreted at an average rate of 21.5 mg. per day, when the intake was only 2 mg. per day. The phosphorus excretion was also greater than the dietary intake, and the serum phosphorus was high.

As a check on this condition, a second group of rats averaging 65 gm. initial weight were fed this diet (5/5 R). The larger animals tolerated the total replacement of calcium no better than the smaller ones. Consequently, 0.5% calcium carbonate was returned to the diet (5/5 Ra) at the end of the first week. The amount of calcium was sufficient to check the rapid loss in weight and to improve the general condition of the animals.

Except in this attempt to replace all of the calcium with manganese, the effect of manganese was more pronounced in relation to phosphorus retention than to that of calcium. Decreasing the calcium to change the calcium: phosphorus ratio also improved mineral assimilation in the presence of manganese. Even when the calcium: phosphorus ratio was reduced so that the diet was no longer rachitogenic, the presence of manganese still induced a rachitic condition.

The administration of vitamin D to animals receiving high manganese diets improved the condition of the animals on all manganese levels, but the effect was most pronounced where the calcium was replaced by manganese. The calcium and phosphorus elimination in the feces was definitely decreased in the presence of vitamin D, and in most cases there was a small gain in weight.

The most striking values in the fecal excretion data for the animals of both the control and the manganese groups are those following the addition of potassium citrate to the diet with the vitamin D supplement. In the animals of the control group and the 2.5 R group the calcium retention was markedly increased; the phosphorus retention was far more than doubled in all three of the groups receiving the citrate (control, 2/5 S, and 2/5 R). The values for phosphorus absorption are given on the assumption that all of the 0.35 to 0.50% phosphorus in the various diets was in available form. Lowe and Steenbock ('36) reported that of the 0.33% total phosphorus in the Steenbock diet only 6% is inorganic, and the rest is in a poorly assimilated form. Hence, the intake values given in these data are maximum. The controls show 23.5 to

32.0% retention of the total phosphorus present in the diet which, according to the work cited, should be about all of the phosphorus available for absorption. Yet with the addition of potassium citrate to the diet, it is notable that almost 75% of the total dietary phosphorus was apparently absorbed. Citrate did not have a marked effect on manganese retention.

Although manganese was evidently instrumental in increasing the fecal excretion of calcium and phosphorus, the feces did not contain all of the ingested manganese. The question arises, was part of the severe condition of the animals caused by the toxicity of the absorbed manganese or by some specific effect on calcium-phosphorus metabolism other than their excretion into the intestine? Marked storage of manganese occurred in the livers of these rats. The amount stored was in proportion to the intake and absorption. It was noted that, although increased phosphorus of the diet (2/5 Rp) resulted in considerable retention of manganese, the amount found in the liver was not as high as from a similar diet without the added phosphorus (2/5 R). The ash of the bones and kidneys contained manganese, but the quantities present in the available samples were too small to allow quantitative measurement by the method used.

In addition to a direct toxic effect of stored manganese, its presence in the tissues may affect the local calcification mechanism in the bone. In the instances of the rats receiving the high manganese diets, both the low blood phosphorus and the results of the line tests indicated a condition of severe rickets. These values, however, are readily explained by the interference with calcium and phosphorus assimilation. With the levels of manganese in the diet that caused a negative phosphorus balance, the phosphorus was drained from both bones and other tissues, and the rachitic condition was probably complicated with osteoporosis. This is especially true with the diet 5/5 R where all of the calcium was replaced with manganese. After only 14 days on this diet, the skeletons of the animals were brittle shells of cortical bone. The epiphyseal line was regular and did not indicate the usual rachitic condition, but the bone had been dissolved from the interior and the marrow cavity filled with blood. The mesothelial linings were hemorrhagic, and the muscles were soft and small. These conditions were undoubtedly caused by the draining of calcium and phosphorus into the intestine. There was nothing which can be interpreted as a specific manganese effect, for the same result could be obtained by maladjustment of the calcium and phosphorus intake alone.

A further indication that the condition was a result of excessive mineral excretion was demonstrated by its improvement with the administration of vitamin D. The general effect of vitamin D was to increase calcium and phosphorus retention and the serum phosphorus level. This increase in phosphorus retention was small but consistent, in spite of the fact that the manganese present was antagonistic to the increased absorption induced by vitamin D. The bones from several groups of animals fed manganese showed some signs of healing along the epiphyseal line, although there was no deposition in the decalcified area of the metaphysis as in normal healing.

Here again the potassium citrate supplement with vitamin D gave significant results as was expected from the improvement in growth and in calcium and phosphorus retention. With the citrate the blood phosphorus was increased and the bones healed to an extent greater than that caused by 100 units of vitamin D alone.

The effects of small amounts of manganese in the diet were not so outstanding (table 2). When added to the milk powder diet, manganese at levels of 25 and 50 p.p.m. showed no appreciable or constant effect on growth, calcium and phosphorus absorption, or blood and bone content. Under these conditions, an average of about 40% of the ingested manganese was apparently absorbed compared to 25% absorption from high manganese intakes when fed with the Steenbock diet. Liver data indicated storage of manganese in proportion to the level in the diet, but the livers of the rats receiving the manganese-free milk powder diet still contained appreciable manganese after 5 weeks' experimental period. About 70% of the total phosphorus was retained compared to 25 to 30% from the Steenbock diet. The blood and bone analyses yielded normal values.

The groups of rats receiving 50 p.p.m. manganese either by injection or in the diet in combination with the Steenbock diet showed a growth rate, slightly but consistently above that of the control animals. On the other hand, with a level of 100 p.p.m. in the diet, the animals showed slightly retarded growth rates compared to the controls. With the exception of a higher food intake for the animals on the 0.005% levels, the calcium and phosphorus retention and blood and bone analyses showed no appreciable differences between the animals receiving manganese and the control group (table 2).

The injection of manganese in an amount equivalent to 100 p.p.m. in the diet retarded the growth rate to a more marked extent. It may also be seen that the phosphorus excretion was increased in these rats. The explanation for the increased fecal excretion of phosphorus may be in the excretion of the injected manganese, in part at least, into the intestine. The fecal excretion of manganese for both groups in which the manganese was injected was several times the amount ingested in the diet. This agrees with reports in the literature on the excretion of manganese in the bile (Reiman and Minot, '20). In the present studies the amount of manganese found in the livers of the animals injected was twice as great as when the same amount was ingested in the diet.

The injected manganese showed little detrimental effect on the healing of the rachitic condition developed on the Steenbock diet. Vitamin D induced healing in all cases. Even with the manganese injections, the bones of two out of the four animals on the 0.005% level and five out of the six on the 0.01% level showed healing. The serum phosphorus of the latter group, however, remained at a rachitic level consistent with the decreased retention of phosphorus in this group.

From the results of the subcutaneous injections of manganese it appears that the rat does not tolerate more than about 50 p.p.m. of manganese in the diet. At levels of 100 p.p.m. the detrimental effect became evident.

SUMMARY

The effects of various amounts of manganese in the diet of young growing rats were studied with special reference to the influence of this element on calcium and phosphorus metabolism in the presence and absence of vitamin D. The following conclusions were drawn from the data obtained.

1. On high manganese intakes the growth of rats was retarded in proportion to the amount of manganese in the diet.
2. High manganese intake in connection with high calcium-low phosphorus diets increased the excretion of phosphorus in all cases and of calcium at the highest manganese levels. This excretion was by way of the feces and in some cases was increased to the extent of depleting the body supply of these elements. The interference with phosphorus retention was more pronounced than was that with calcium retention.
3. The adverse nutritional condition was less marked when the calcium in the diet was replaced than when it was supplemented by manganese. When the calcium of the diet was completely replaced by manganese, the results were fatal because of the severe loss of calcium and phosphorus from the body.
4. A more favorable calcium: phosphorus ratio in the diet, produced by increasing the phosphorus content, tended to improve retention of these elements even in the presence of manganese.
5. Animals from these groups which had been fed large amounts of manganese developed severe rickets as evidenced by blood phosphorus level and by the condition of the bones. Vitamin D administration resulted in an improved condition.
6. The addition of potassium citrate to the diet produced a striking improvement in calcium and phosphorus retention and subsequently in bone calcification.
7. Manganese was stored primarily in the liver. That which entered the body by subcutaneous injection was stored to some extent in the liver and the bones, but the larger portion was excreted in the feces.
8. Low manganese intakes, comparable to those found effective in correcting perosis in the chick, did not retard

growth and showed little or no effect on bone calcification either in conjunction with normal or with rachitogenic diets.

LITERATURE CITED

BARNES, L. L., G. SPERLING AND L. A. MAYNARD 1941 Bone development in the albino rat on a low manganese diet. Proc. Soc. Exp. Biol. Med., vol. 46, p. 562.

BLUMBERG, H., D. H. SHELLING AND D. A. JACKSON 1938 The production of manganese rickets in rats. J. Nutrition, vol. 16, p. 317.

CLARK, E. P., AND J. B. COLLIP 1925 A study of the Tisdall method for the determination of blood serum calcium with suggested modifications. J. Biol. Chem., vol. 63, p. 461.

DELSAL, J. L. 1938 A polarimetric and electrometric study of the tartaric and malic complexes of the bivalent metals. J. chim. phys., vol. 35, p. 314.

GALLUP, W. D., AND L. C. NORRIS 1939 a The amount of manganese required to prevent perosis in the chick. Poul. Sci., vol. 18, p. 76.

——— 1939 b The effect of a deficiency of manganese in the diet of the hen. Poul. Sci., vol. 18, p. 83.

HATHAWAY, M. L., AND F. L. MEYER 1939 Citric acid and its salts as calcifying agents in rats. J. Nutrition, vol. 17, p. 419.

HILLEBRAND, W. F., AND G. E. F. LUNDELL 1929 Applied Inorganic Analysis. John Wiley & Sons, Inc., New York.

LOWE, J. T., AND H. STEENBOCK 1936 Cereals and rickets. VII. The role of inorganic phosphorus in calcification on cereal diets. Biochem. J., vol. 30, p. 1126.

ORENT, E. R., AND E. V. MCCOLLUM 1931 Effect of deprivation of manganese in the rat. J. Biol. Chem., vol. 92, p. 651.

REIMAN, C. K., AND A. S. MINOT 1930 Absorption and elimination of manganese ingested as oxides and silicates. J. Biol. Chem., vol. 45, p. 133.

SHOHL, A. T. 1937 The effect of the acid-base content of the diet upon the production and cure of rickets with special reference to citrates. J. Nutrition, vol. 14, p. 69.

SKINNER, J. T., AND W. H. PETERSON 1930 The determination of manganese in animal material. J. Biol. Chem., vol. 88, p. 347.

WILLARD, H. H., AND L. H. GREATHOUSE 1917 The colorimetric determination of manganese by oxidation with periodate. J. Am. Chem. Soc., vol. 39, p. 2386.

YOUNGBERG, G. E., AND M. V. YOUNGBERG 1930 A system of blood phosphorus analysis. J. Lab. Clin. Med., vol. 16, p. 461.

FEEDING-CANE MOLASSES AS A CONSTITUENT OF POULTRY RATIONS. I. MOLASSES FOR GROWING CHICKENS

BY

WALTHER H. OTT, R. V. BOUCHER AND H. C. KNANDEL

Reprint from POULTRY SCIENCE: Vol. XXI, No. 4
July, 1942

Feeding-Cane Molasses as a Constituent of Poultry Rations.* I. Molasses for Growing Chickens

WALTHER H. OTT AND R. V. BOUCHER
Department of Agricultural and Biological Chemistry
AND
H. C. KNANDEL
Department of Poultry Husbandry, The Pennsylvania State College, State College, Pennsylvania

(Presented at annual meeting August, 1941; received for publication October 25, 1941)

FEEDING-CANE molasses has been used to a limited extent in poultry rations, although for many years it has been an important constituent of rations of other farm animals. It is usually one of the most abundant as well as one of the cheapest sources of energy available to poultrymen in this country.

Winter (1929) reported on four trials with chicks grown in batteries and noted that eight-week body weights were highest in groups receiving 5 percent cane molasses replacing an equal amount of corn in the ration. In one trial continued to 12 weeks, body weight was highest in the lot receiving 3 percent molasses. Maw (1933) added molasses to a starting mash to form 1, 3, 5, and 7 percent of the total ration and found in one 10-week battery trial that body weight was essentially constant while feed intake increased with increased molasses in the ration.

Bice (1933) fed a commercial mash to which was added 5, 7, and 10 percent molasses. On the basis of the additional consumption of scratch grain, chopped green alfalfa, and bonemeal reported for the 13-week experiments with chicks reared on the floor, molasses apparently constituted about 3.4, 4.9, and 6.1 percent of the total rations. From Bice's data it is noted that, after the sixth week, growth curves of females fed 3.4 and 4.9 percent molasses were close together and higher than growth curves of females fed 6.1 percent and no molasses. Total feed intake was highest in the groups receiving molasses.

In seven 8-week experiments with chicks in batteries, Upp (1937) observed inferior growth, higher feed intake, and lower feed efficiency with diets containing 5, 10, and 15 percent molasses in place of an equal amount of corn. The 15 percent molasses diet was too laxative for most of the chicks, and the recommendation was made that not more than 5 to 7 percent molasses be used in all-mash chick rations.

Evidently a small amount of cane molasses may be used satisfactorily in the chick diet. In order to obtain information relative to the optimum amount of molasses for practical poultry rations, an investigation was conducted in which all-mash rations containing 0, 2, 4, and 6 percent feeding-cane molasses were fed through the entire growing period and the first laying year. The all-mash no-molasses basal ration was formulated to meet all known nutritive requirements with constituents conforming to best present-day commercial practice in this area. Thus, the investigation was designed to indicate whether or not the addition of feeding-cane molasses would improve an already high-grade ration.

TABLE 1.—*Mean body weight of females*

Age	Percent cane molasses			
	None	2	4	6
weeks	gms.	gms.	gms.	gms.
1	64	63	63	63
4	211	207	219	207
8	501	504	523	503
12	866	862	887	868
16	1169	1164	1187	1158
20	1370	1363	1384	1379
24	1595	1607	1602	1632
Number	149	156	147	160

EXPERIMENTAL

Data reported here were obtained in the first 24 weeks of two experiments in which four all-mash rations varying in feeding-cane molasses content were fed in duplicate to Single Comb White Leghorns from 1 day to 72 weeks of age. The first experiment was started April 26, 1939, and the second May 8, 1940. The molasses was obtained as a composite sample from the 1938 season's production of 14 Cuban sugar mills.

TABLE 2.—*Mean feed intake of females*

Age	Percent cane molasses			
	None	2	4	6
weeks	lbs.	lbs.	lbs.	lbs.
1– 4	1.15	1.16	1.23	1.20
4– 8	3.02	3.05	3.24	3.08
8–12	3.84	3.77	4.12	3.90
12–16	4.40	4.46	4.67	4.50
16–20	4.76	4.99	5.04	5.20
20–24	5.33	5.49	5.73	5.96
Total	22.50	22.92	24.03	23.84

TABLE 3.—*Mean feed efficiency of females expressed as pounds of gain per pound of feed consumed*

Age	Percent cane molasses			
	None	2	4	6
weeks				
0– 6	0.266	0.265	0.263	0.259
6–12	0.210	0.208	0.197	0.208
12–24	0.112	0.107	0.101	0.110

The no-molasses basal ration contained ground yellow corn 42.3, wheat bran 15, standard wheat middlings 10, ground oats 10, alfalfa meal 5, meat scraps 7, fish meal 7, dry skimmilk 2, ground limestone 1, salt 0.5, and cod liver oil (400-D) 0.2 percent by weight. Mean chemical analyses of this ration were moisture 10.3, protein 17.9, and ash 7.4 percent. At 16 weeks of age this ration was changed to contain ground yellow corn 45.1, wheat bran 15, standard wheat middlings 10, ground oats 10, alfalfa meal 5, meat scraps 5, fish meal 5, dry skimmilk 2, ground limestone 2, salt 0.5, and cod liver oil (400-D) 0.4 percent by weight. Mean chemical analyses of this ration were moisture 10.9, protein 14.7, and ash 6.9 percent. Ground yellow corn in the basal diet was replaced by 2, 4, and 6 percent feeding-cane molasses to make the three molasses-containing diets.

TABLE 4.—*Percent mortality of females*

Exp.	Age	Percent cane molasses			
		None	2	4	6
	weeks	%	%	%	%
1	0–12	6.2	9.6	6.6	5.4
1	12–24	2.6	2.4	2.0	2.6
1	6*	5.3	8.8	6.6	1.7
1	20*	8.8	2.4	1.5	1.5
2	0–12	0.8	2.0	1.5	1.5
2	12–24	5.4	0	0	0

* Females were removed at these ages because of emaciation.

The molasses was incorporated into the ration by first adding the required amount

---

* Authorized for publication on October 20, 1941, as paper No. 1056 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This investigation was made possible through a fellowship grant by the Commercial Molasses Corporation, New York City.

TABLE 5.—*Mean body weight of males*

Age	Percent cane molasses			
	None	2	4	6
weeks	gms.	gms.	gms.	gms.
1	67	66	65	66
4	231	232	236	233
8	531	538	563	549
12	956	923	920	942
Number	181	167	182	179

TABLE 6.—*Mean feed intake of males*

Age	Percent cane molasses			
	None	2	4	6
weeks	lbs.	lbs.	lbs.	lbs.
1– 4	1.15	1.16	1.23	1.20
4– 8	2.72	2.65	2.96	2.87
8–12	3.98	3.80	4.11	4.00
Total	7.85	7.61	8.30	8.07

of the cold, undiluted molasses to six times its weight of ground yellow corn. The mixture was shoveled back and forth a few times on the floor and then stored in burlap bags for two days. After standing that length of time the mixture was ground in a hammer mill using a three-sixteenths inch mesh screen. No difficulty was experienced with sticking and a granular product resulted, not appreciably finer than the untreated ground corn itself. Rations were mixed at approximately monthly intervals. Under this procedure the moisture contents of the four rations were essentially equal at time of mixing.

TABLE 7.—*Mean feed efficiency of males expressed as pounds of gain per pound of feed consumed*

Age	Percent cane molasses			
	None	2	4	6
weeks				
0– 6	0.266	0.265	0.263	0.259
6–12	0.240	0.230	0.214	0.222

Started in the first experiment were 936 day-old pedigreed chicks from yearling hens and 64 non-pedigreed chicks from pullets of The Pennsylvania State College breeding flock. Each hen's chicks were taken in turn and distributed at random among as many as possible of the eight pens. For the second experiment 630 pedigreed chicks were obtained from yearling hens of the college breeding flock, and 301 pedigreed and 66 non-pedigreed chicks were obtained from pullets in the first experiment. These latter 367 chicks were fed the same diets their parents had received and were used to furnish information regarding performance of a second generation on the respective diets.

At six weeks of age all males were transferred to wire growing batteries where they were fed the same diets they had been receiving, and were removed from the experiment at 12 weeks of age. No attempt was made in the first experiment to equalize the numbers of females in the various pens prior to the beginning of the laying period or the numbers of males placed in the battery cages. In the second experiment a total of 74 pedigreed males at 6 weeks and a total of 50 pedigreed females at 20 weeks of age were removed to equalize the numbers in the several pens at those times. All non-pedigreed males in the second experiment were removed at the sixth week, and the non-pedigreed females were removed at the twentieth week.

TABLE 8.—*Percent mortality of males*

Exp.	Age	Percent cane molasses			
		None	2	4	6
	weeks	%	%	%	%
1	0– 6	5.4	4.5	5.8	3.1
1	6–12	3.1	2.7	2.1	3.7
2	0– 6	4.0	7.4	4.2	3.3
2	6–12	0.9	0	0	1.8

Chicks were weighed individually and feed intake per pen was recorded at weekly

intervals from 1 to 12 weeks of age and at biweekly intervals thereafter. The chicks were brooded and reared in a continuous type poultry house, care and management practices being kept as uniform as possible.

Females

Mean body weights on the 4 percent diet were higher than those on the other diets from the second to the twenty-second week, the difference being statistically significant only at the eighth week (see also Table 10). The superiority of the 4 percent group over the next highest group was 3.6 percent at 4 weeks, 3.7 percent at 8 weeks, and 2.3 percent at 12 weeks of age.

Growth curves for the 0, 2, and 6 percent groups were comparatively close together after the sixth week. Mean body weights on the 2 and 6 percent diets were lowest up to the sixth week. A study of relative growth rates in different phases of the growing period indicated that from one to four weeks of age, growth on the 4 percent diet was about 5 percent faster than on any of the other diets. Also from 16 to 24 weeks, growth on the 6 percent diet was 7 percent greater than on the other diets.

Total 24-week feed intakes on the 4 and 6 percent diets were significantly greater than those on the 0 and 2 percent diets. Mean feed intake on the no-molasses diet was lowest during all phases of the growth period except the interval from 8 to 12 weeks of age.

Mean feed efficiency for increase in body weight throughout the growing period was lowest in the molasses fed groups. Differences were statistically significant only for the period from 12 to 24 weeks of age, when the feed efficiencies on the 2 and 4 percent diets were respectively 5 and 10 percent below that on the no-molasses diet.

A rather severe coccidiosis infestation occurred early in the first experiment, which contributed largely to the high mortality. Beta lactose, mixed into enough diet to make a one day supply of a 15 percent lactose diet, was used at weekly intervals up to the twelfth week. At 6 weeks of age a total of 26 extremely emaciated females were removed for autopsy, and at 20 weeks 21 more females were removed because of the same condition. Examination showed essentially the same frequency of disorders as was found in the cases of mortality. The use of beta lactose at this time was the only instance in the entire experimental work in which the birds had access to any other food material than the experimental all-mash diets and an *ad libitum* supply of oyster shell, grit, and water.

No disease outbreaks occurred in the second experiment, and mortality was relatively low. Although total losses in both experiments were lowest on the two high molasses diets, the mean differences between diets in this respect were not significant.

Males

Males fed 4 percent molasses in the diet were highest in mean body weight from 2 to 10 weeks of age, inclusive. The superiority was greatest at the eighth week, when this group was 6 percent heavier than the no-molasses group. Mean body weight at 12 weeks of age was greatest on the no-molasses diet.

Rates of growth on the diets containing molasses were higher from 1 to 8 weeks of age and lower from 8 to 12 weeks of age than the corresponding growth rates on the no-molasses diet. However, differences in mean body weight and in growth rates were not statistically significant.

Feed intakes on the 4 and 6 percent diets were consistently higher than those on the 0 and 2 percent diets, the differences in total 12-week feed intake being significant. Mean feed efficiencies on the molasses diets were below those on the no-molasses diet, the differences between diets being signifi-

TABLE 9.—*Mean body weights of females in 1939 and their progeny in 1940*

Age	Percent cane molasses							
	None	2	4	6	None	2	4	6
	Dams				Female progeny			
weeks	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
1	67	63	68	68	65	62	65	63
4	237	215	242	220	209	185	219	193
8	559	515	544	517	512	461	546	490
12	941	875	916	921	881	795	895	845
16	1228	1176	1213	1240	1208	1119	1207	1192
20	1454	1463	1434	1549	1420	1326	1405	1413
24	1722	1624	1711	1829	1594	1534	1591	1703
Number	11	11	14	9	33	22	33	33
Age	Dams				Male progeny			
1	67	64	67	67	68	65	67	66
4	233	218	241	221	231	205	243	213
8	554	530	542	514	554	485	613	515
12	933	891	913	922	1004	843	981	906
Number	13	10	13	11	33	36	45	44

cant in the period from 6 to 12 weeks of age. In this period the no-molasses group was highest and the 4 percent group was lowest in feed efficiency, the difference being almost 11 percent.

Mortality was essentially equal on the four diets. No significant relationship has been observed between the amount of molasses in the diet and the incidence of any of the diagnosed causes of mortality among either the males or the females.

No differences between diets were noticeable in the outward appearance of the birds. The laxative effect of the 6 percent molasses diet was very slight, and did not present any practical difficulties with regard to dryness of litter on the floor.

Second Generation

In Table 9 is shown the comparative growth of certain females in the first experiment and their female and male progeny on the same diets in the second experiment. The 1939 females selected were those with highest egg production and fertility. Only eggs weighing 52 grams and over were used. This method of selection accounts partly for the variation in initial weight and numbers of progeny.

With few exceptions the mean weights of female progeny were below, but quite close to, the corresponding weights of their parents until about the sixteenth week of age. The smallest difference in growth between the two generations occurred on the 4 percent diet. The characteristic rank of the female progeny on the four diets was essentially identical with that of their parents throughout the growth period. At 24 weeks of age the 6 percent diets were highest, the 4 and 0 percent diets intermediate, and the 2 percent diets lowest in body weight.

The growth curve of the male progeny on the 6 percent diet was almost the same as that of their female parents. From 8 to 12 weeks of age mean body weights of the male progeny on the 0 and 4 percent diets were greater than those of their female parents, while weights of male progeny on the 2 percent diet were less than those of their dams. Males on the 0 and 4 percent diets were heaviest at 12 weeks of age.

TABLE 10.—*Summary of criteria in which dietary differences were significant, showing significance of differences between diet means*

Criterion	Age in weeks	F (anal. of var.)	Percent cane molasses in diets compared					
			0:2	0:4	0:6	2:4	2:6	4:6
Females								
Mean body weight	8	S	—	HS	—	HS	—	HS*
Mean feed intake	1– 4	HS	—	HS	S	HS	S	—
Mean feed intake	1–24	HS	—	HS	HS	HS	HS	—
Mean feed efficiency	12–24	HS	—	HS*	—	HS*	S	HS
Males								
Mean feed intake	1–12	S	—	S	—	HS	S	—
Mean feed efficiency	6–12	S	—	S*	S*	—	—	—

* In these five instances of significant differences between diet means, the lower molasses diet had the higher value. In the remaining 15 instances the higher molasses diet had the higher value. S(significant) and HS (highly significant) refer to significance at the points of probability of 0.05 and 0.01, respectively. Dashes indicate non-significant differences.

Mortality among the female progeny was 0, 7.8, 2.9, and 0 percent on the 0, 2, 4, and 6 percent diets, respectively. Mortality among the males was 2.9, 7.7, 4.2, and 6.4 percent on the 0, 2, 4, and 6 percent diets, respectively. No reasons were evident for the higher mortality in the 2 percent females or the 2 and 6 percent males.

DISCUSSION

Statistical analyses were conducted on each criterion of performance, consideration being given to essentially all possible arrangements and combinations of the data. In only six instances, listed in Table 10, were significant differences found between diet means. In all other criteria the mean performances on the four diets were within the limits of normal variation.

With regard to both males and females, analyses of variance have shown that in almost all criteria in these experiments the relative performance on the four diets in one series of four pens was not significantly different from that in the duplicate series. Furthermore, in most criteria the relative performance on the four diets in the first experiment was not significantly different from that in the second experiment. For these reasons, mean values for the four pens on each diet were given in the tables.

SUMMARY

Statistical analyses of the results of two experiments involving 746 females up to 24 weeks of age and 867 males up to 12 weeks of age showed that, in most criteria, the mean performances on all-mash diets containing 0, 2, 4, and 6 percent feeding-cane molasses were within the limits of normal variation.

The evidence seems to indicate that the 4 percent cane molasses diet had a slight early-growth stimulative effect upon both sexes. Growth of the second generation on the 4 percent diet was relatively slightly superior to the growth of second generations on the other diets.

Total feed intake for the growing period was increased significantly by the inclusion of 4 and 6 percent cane molasses. Feed efficiency was highest on the no-molasses diet.

REFERENCES

Bice, C. M., 1933. Cane molasses in poultry rations. Hawaii Agr. Exp. Sta. Bul. 67.

Maw, A. J. G., 1933. Feeding cane molasses to growing chicks and laying hens. Sci. Agr. 13: 743-745.

Upp, C. W., 1937. Cane molasses in poultry rations. Louisiana Agr. Exp. Sta. Bul. 289.

Winter, A. R., 1929. Cane molasses for poultry. Poultry Sci. 8:369-373.

Deposition of Lead from Lead Arsenate Spray Mixtures and Its Retention on Pyralin Plates and Apple Leaves and Fruits[1]

H. N. Worthley and D. E. H. Frear, *Pennsylvania State College, State College, Pa.*[2]

The desirability of a rapid laboratory method by which the deposition and retention of a given spray mixture may be quickly evaluated is obvious. Such a method, if it should correlate reasonably well with field results, would enable the investigator to test a relatively large number of possible combinations of spray materials, and, by elimination, select for field testing those giving the greatest deposit and the highest degree of retention of the active constituents.

Various laboratory methods for testing sprays have been devised. McCallan & Wilcoxon (1940) have discussed at some length the theoretical basis for such tests as applied to fungicides, as have Horsfall *et al.* (1940). Hensill & Hoskins (1935), Hoskins & Wampler (1936) and Ben-Amotz & Hoskins (1937) have devised laboratory methods for testing the deposition and retention of insecticides in order to study certain of the fundamental factors involved in the process. In these investigations the laboratory work has not been correlated with field studies. Dawsey *et al.* (1937), however, have made a comparison of laboratory and field results in their study of oil deposits. Apparently little work has been done on the correlation of laboratory and field investigations in the case of one of the most commonly used orchard insecticides, lead arsenate.

In the present paper the authors report studies of relative deposition and retention of lead in a series of spray mixtures containing lead arsenate. Results secured in the laboratory are compared with field records of the use of the same mixtures on apple foliage and fruits.

Laboratory Spray Apparatus and Procedure.—The spraying device used in the laboratory is shown in figure 1. Essentially it consisted of a turntable of heavy plywood 48 inches in diameter connected through a reducing gear to a constant speed motor of the synchronous type, to give a speed of 14 revolutions per minute. On the upper surface at the periphery of the turntable were placed 22 wooden blocks 4 inches high and approximately 6 inches wide. The outer surface of each block was so beveled as to present a rectangular surface approximately 5 by 6 inches at an angle of 45 degrees to the horizontal table surface. The spray was applied by a large commercial atomizer type sprayer, such as is used in automobile painting, directed at right angles to the sprayed surfaces, and at a distance of approximately 24 inches. The liquid to be sprayed was contained in a beaker immediately below the atomizer, and kept under continuous agitation by means of an electric stirrer. The air pressure used in these experiments was kept constant at 120 pounds per square inch. The room in which the apparatus was located was kept at a nearly constant temperature and relative humidity.

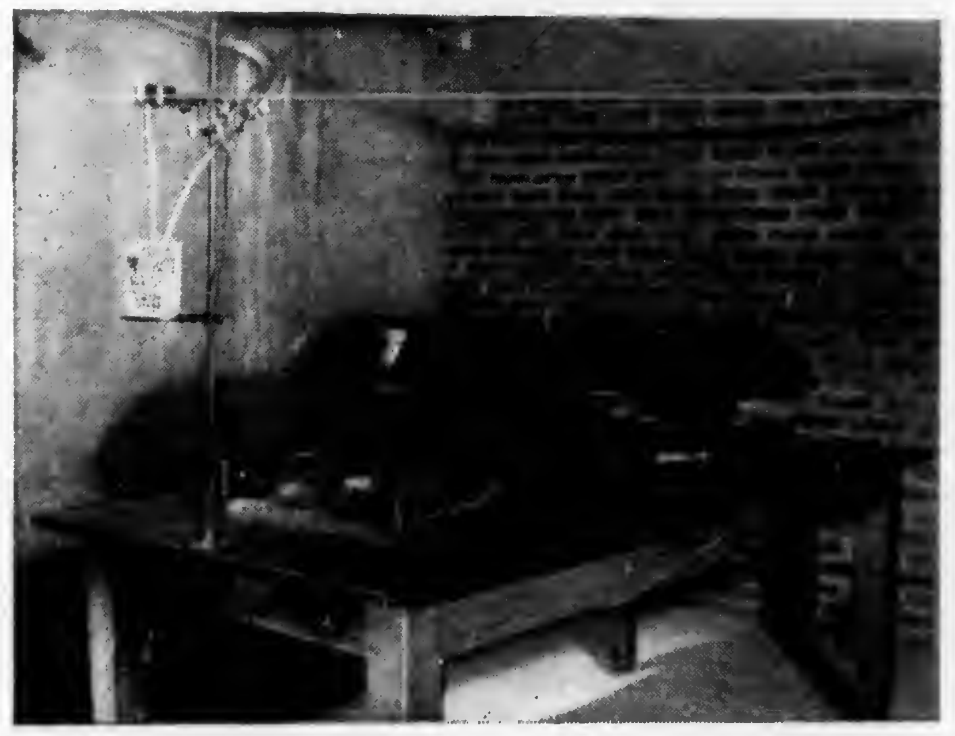


Fig. 1.—Spray turntable assembly used to study spray deposit and retention on *Pyralin* plates.

The surfaces upon which the liquids were sprayed were cut from sheets of *Pyralin*, a cellulose nitrate plastic film manufactured by the E. I. duPont de Nemours Co. *Pyralin* was chosen for this study after an investigation of a number of synthetic surfaces. A macroscopic examination of the deposits produced on this surface indicate a close similarity to those produced on certain natural plant surfaces. The film, which was 0.0125 inch thick, was cut into plates 5 inches square, one of which was pinned to each beveled surface of 20 of the blocks on the turntable. The plates were always handled with rubber gloves prior to spraying and by the edges after spraying. All plates were cut from the same manufacturer's lot of material.

---

[1] Authorized for publication on December 5, 1941, as Paper No. 1066 in the Journal of the Pennsylvania Agricultural Experiment Station.

[2] Professor of Economic Entomology and Assistant Professor of Agricultural and Biological Chemistry, respectively.

In spraying the turntable was started and the atomizer was turned on, the first spray being caught in a larger beaker. After a few seconds the beaker was quickly removed, and the spray allowed to strike the rotating plates. Rather than adopt a standard spraying time, or a standard amount of spray material, spraying was continued until the deposited drops started to coalesce (single dosage). The number of revolutions of the turntable necessary to produce this effect varied with the different mixtures employed. With a second series of applications, spraying was continued through twice the number of revolutions used in the first series (double dosage), in order to study the effect of overspraying. The plates were allowed to dry, and were then removed and hung in a protected place for 15 days. At the end of this time, 15 of the plates were placed on the turntable as before, rotated, and sprayed with 250 ml. of distilled water. After drying, five of the plates were removed and the remaining ten sprayed with an additional portion of 250 ml. of water. Of the original 20 plates, 5 received no washing, 5 received 250 ml. of water, 5 received 500 ml., and 5 received 750 ml. The exact amount of wash water per unit area of the plates was not determined.

After the washing all plates were trimmed to exactly 10×10 cm. in size and thoroughly washed with hot nitric acid (1+1). The washings from each plate were made to a definite volume and were analyzed for lead. Figures reported are averages of duplicate tests of both single and double dosages.

FIELD SPRAY APPARATUS AND PROCEDURE.—In the field, 14 mature Stayman Winesap trees were selected. Each was sprayed with one of the mixtures as used in the laboratory. The sprays were applied with a single-nozzle spray gun from a power sprayer that delivered 10 gallons per minute at about 350 pounds pressure per square inch. To minimize the effect of drip from above the zone in which leaf and fruit samples were taken, the application was confined to the lower branches 10 feet or less above the ground. These were sprayed thoroughly, and with a special effort at uniformity, both from inside and outside the foliage wall. However, the mixtures containing deposit builders were not applied in greater amounts than the others. Some run-off occurred from each tree.

With a specially designed leaf punch 100 discs were cut from leaves near the center of current season twig growth selected at random about the periphery of each tree. Each disc had an area of 11 square centimeters. Samples were taken on July 23 before and after spraying. To reveal the degree of retention, further samples were taken on August 5, August 13, August 18 and September 10, after precipitation totaling 0.91, 1.23, 3.30, and 7.25 inches on the respective dates. The samples were dried at 100° C., weighed and ground. Aliquot portions of the dried leaves were ashed, the ash dissolved in nitric acid and analyzed for lead.

Fruit samples of 30 apples each were taken at the same time as the leaf samples. These apples were cored with a cork borer, and the remainder of the fruit peeled. The peels from the fruit surfaces (excluding the stem and calyx ends removed with the cores) were digested with nitric and sulfuric acids, and when all organic matter was removed were made to volume and analyzed for lead.

ANALYTICAL PROCEDURE.—The samples were analyzed for lead by the sulfide method in essentially the same manner as described by Frear and Haley (1934). An Evelyn photoelectric colorimeter was used, with a filter transmitting in the neighborhood of 400 millimicrons.

SPRAY MIXTURES USED.—The composition of the various mixtures used in this experiment is given in table 1. All were selected because of interest in their use in apple spray schedules in the eastern states. In preparing the homemade "dynamite" sprays—treatments 13 and 14, mixtures A and B were beaten together to form a creamy emulsion. This was poured into the spray suspension to give the final dilution, and was sprayed out with as little agitation as possible. In the field, too much agitation in the spray tank caused

the lead arsenate particles in treatment 13 to flocculate into relatively large clumps. As spraying proceeded, these clumps adhered to the sides of the spray tank, and to the agitator shaft and blades, and served to reduce greatly the deposition of lead arsenate on the trees. The presence of bordeaux mixture in treatment 14 prevented the formation of large flocs.

LABORATORY STUDIES.—The *Pyralin* plates sprayed and washed in the laboratory as described above, when analyzed for lead, gave results as shown in figure 2. The amounts deposited by the different spray mixtures ranged from 66 to 455 milligrams per square meter of plate surface, and thus fell within the limits reported in earlier publications (Frear & Worthley, 1937, 1940) for spray applications to apple foliage and fruits.

In treatments 1 through 4 no deposit builders were used. Overspraying with lead arsenate alone however (treatment 1) produced the greatest buildup of any mixture employed, although the deposit produced was readily removed by washing. A striking effect was noted when liquid lime sulfur and hydrated lime were added to the spray mixture (treatment 2). Not only was the initial deposit extremely low, but overspraying resulted in an actual loss of material. Manganese sulfate and skim-milk powder added to the lead arsenate-lime sulfur mixture gave a relatively low initial deposit of lead, considerable buildup through overspraying, and excellent retention. With bordeaux mixture (treatment 4) the original deposit was low and the buildup negligible, although retention remained excellent.

Proprietary deposit builders were employed in treatments 5 through 12, being used with lead arsenate alone in treatments 5, 8, and 10. Two of these materials gave a high initial deposit, marked buildup in overspraying, and excellent retention. The third was relatively poor. With one deposit builder the addition of finely divided sulfur (treatment 6) appeared to increase the initial deposit and decrease the buildup slightly, but showed little effect upon retention. The addition of bordeaux mixture (treatments 7, 9, 11) in all cases interfered with the action of the deposit builder. The interference with buildup and retention was most pronounced where *Copper Hydro* and lime were used (treatment 12).

Table 1.—Composition of spray mixtures used in laboratory and field.

TREATMENT NUMBER	MATERIALS	AMOUNTS PER 100 GALLONS
1	Acid lead arsenate	3.0 lbs.
2	Acid lead arsenate	3.0 lbs.
	Hydrated lime	5.0 lbs.
	Liquid lime sulfur	1.3 gals.
3	Acid lead arsenate	3.0 lbs.
	Skim milk powder	0.5 lb.
	Manganese sulfate	0.25 lb.
	Liquid lime sulfur	1.3 gals.
4	Acid lead arsenate	3.0 lbs.
	Skim milk powder	0.5 lb.
	Powdered copper sulfate	2.0 lbs.
	Hydrated lime	4.0 lbs.
5	Acid lead arsenate	3.0 lbs.
	Orthex	1.0 qt.
6	Acid lead arsenate	3.0 lbs.
	Mike sulfur	2.0 lbs.
	Orthex	1.0 qt.
7	Acid lead arsenate	3.0 lbs.
	Powdered copper sulfate	2.0 lbs.
	Hydrated lime	4.0 lbs.
	Orthex	1.0 qt.
8	Acid lead arsenate	3.0 lbs.
	Spraylastic	1.5 pts.
9	Acid lead arsenate	3.0 lbs.
	Powdered copper sulfate	2.0 lbs.
	Hydrated lime	4.0 lbs.
	Spraylastic	1.5 pts.
10	Acid lead arsenate	3.0 lbs.
	Colloidal 77-C	0.25 lb.
	Orthol-K	2.0 qts.
11	Acid lead arsenate	3.0 lbs.
	Colloidal 77-C	0.25 lb.
	Orthol-K	2.0 qts.
	Powdered copper sulfate	2.0 lbs.
	Hydrated lime	4.0 lbs.
12	Acid lead arsenate	3.0 lbs.
	Colloidal 77-C	0.25 lb.
	Orthol-K	2.0 qts.
	Copper Hydro	1.0 lb.
	Hydrated lime	4.0 lbs.
13	Acid lead arsenate	3.0 lbs.
	A { Oleic acid	100 ml.
	28% ammonium hydroxide	150 ml.
	Water	1000 ml.
	B { Fuel oil	3.0 pts.
	Menhaden fish oil	1.0 pt.
14	Acid lead arsenate	3.0 lbs.
	Powdered copper sulfate	2.0 lbs.
	Hydrated lime	4.0 lbs.
	A and B as in treatment 13	

A home made deposit builder was used with lead arsenate alone in treatment 13. The initial deposit compared well with the mixtures that employed proprietary deposit builders, and retention was even better. The buildup through overspraying, however, was relatively slight. The addition of bordeaux mixture (treatment 14) reduced the initial deposit of lead somewhat, but appeared to improve the buildup, and did not interfere with retention.

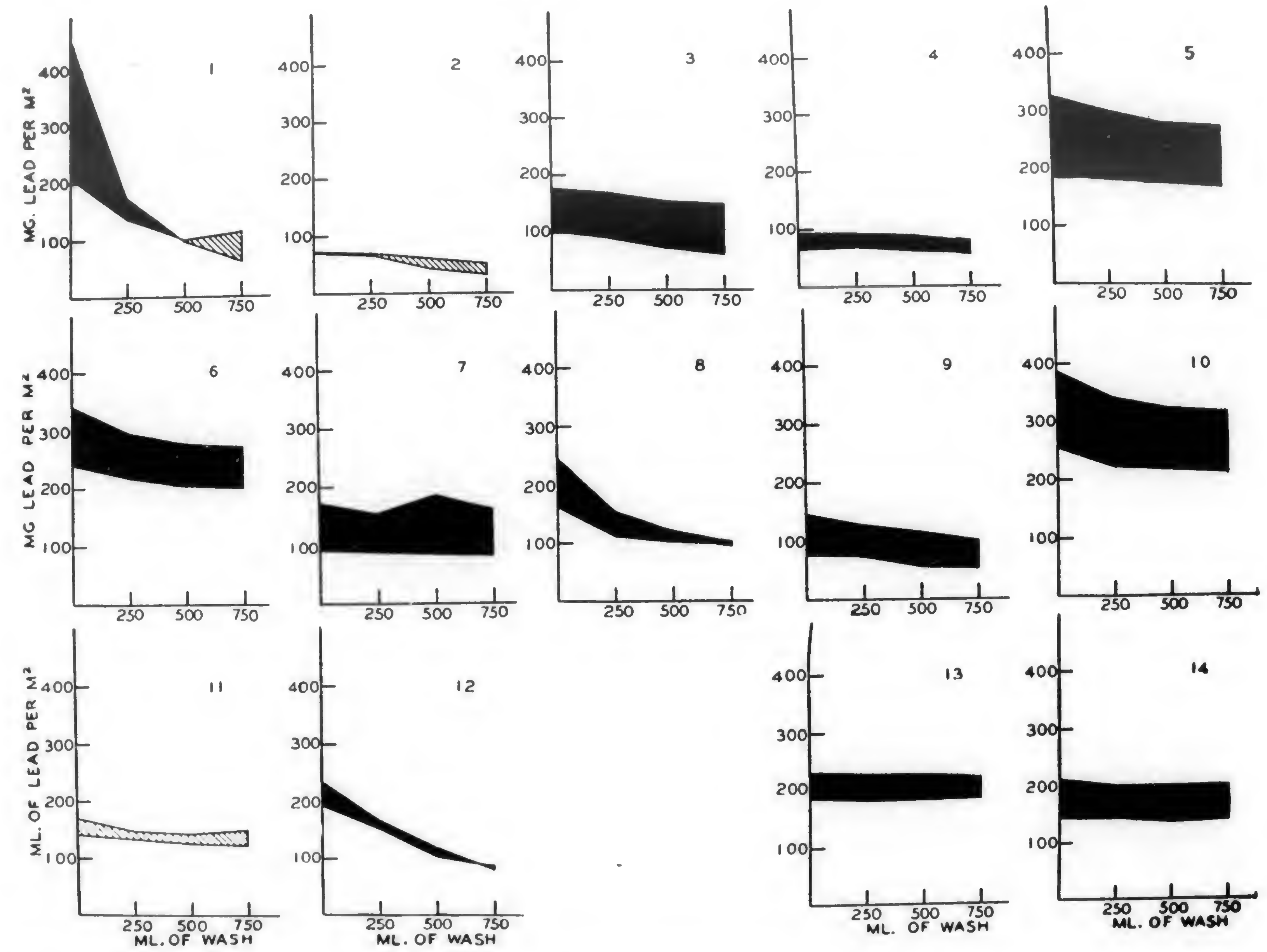


FIG. 2.—Deposition and retention of lead in laboratory spray treatments. In each figure vertical height at left margin indicates extent of buildup with double dosage. Slope of upper and lower edges shows, respectively, the rate of loss from artificial rainfall from double-sprayed and single-sprayed plates. Cross-hatching indicates lower amount of lead on double-sprayed plates.

FIELD STUDIES.—The trees available for the study bore a light crop of fruit, and had already received a complete schedule of sprays, the last, of lead arsenate and weak bordeaux mixture, applied in late June. The experimental treatments were applied nearly a month later, on July 23. At this time residues were determined on the leaves by taking a sample for analysis from each tree before the experimental treatment. Thus it was possible to determine the amount of lead deposited by each of the experimental spray mixtures, and to apply to this figure the per cent retention as calculated from the analysis of samples taken after successive increments of rainfall.

An original intention not to include fruits in this study was reversed after the spray application, when arrangements were made for the additional analyses, and the crop on most trees was deemed adequate to supply a few comparable samples. Lead residues on the fruits were not determined before spraying. Figures for the fruit analyses were calculated on the basis of the average fruit area after the experimental spray application, at the time of the first sampling. This gave results of the same order as calculating the deposit in terms of the amount per fruit, but had the advantage of presenting the data in a form comparable to the leaf and *Pyralin* plate analyses, with the apparent loss due to fruit growth excluded.

Figures derived from the leaf and fruit samples taken in the field, and treated to make them comparable with those obtained in the laboratory, appear in table 2. For comparison, the analyses of the oversprayed plates are included.

Considering the laboratory method as a means of discovering spray mixtures that will prove to be superior in field trials, it is important to examine the order in which the treatments rank with respect to the deposition and retention of lead on the three surfaces employed in this study. Treatments that rank among the first five in both deposition and retention on the plates are Nos. 5, 6, and 10 (table 2). These treatments are also among the first five on the leaves in both points. Treatment 10 gave a high ranking deposit on the fruit, but lost rank in retention on this surface. Treatment 6 gave the best deposit and highest degree of retention on the fruits. The close correspondence between treatment 6 and treatment 5 on both plates and leaves might justify the assumption that lead analyses for treatment 5 on the fruits, samples of which were accidentally destroyed during digestion, would have ranked among the first five. Treatments 1 and 8 showed a high deposit of lead on the plates, but relatively poor retention. Neither treatment appears among the leading five on leaves or fruits.

Similarly, when judged by both criteria, at least three treatments that gave poor results in the laboratory (Nos. 1, 2, and 4) failed to do well in the field. Thus the comparisons allow it to be stated with some confidence that lead arsenate alone or in mixture with liquid lime sulfur and lime or copper sulfate and lime produces relatively inferior lead deposits; that very satisfactory lead levels may be reached and maintained through use of certain proprietary deposit builders, when these are used with lead arsenate alone or with lead arsenate and wettable sulfur; but that the deleterious effect of bordeaux mixture on the lead deposit from these mixtures, as revealed in the laboratory tests is not certain as judged by the field results. The good qualities of some treatments were not discovered in the laboratory tests, and there were others whose behavior was quite different on the three surfaces studied. Treatment 11 is a good example. Lead deposit from this treatment was very poor on the plates, and superior retention raised it in rank only to the middle of the series. The performance of this mixture was distinctly better on the leaves, and it ranked among the first five on the fruits. Treatment 7 is another that in most determinations ranked higher on leaves and fruits than on the plates.

Table 2.—Deposition and retention of lead from lead arsenate spray applications on *Pryalin* plates, and on leaves and fruits.

PLATES		LEAVES		FRUITS	
Treatment	Mg. per Sq. M.	Treatment	Mg. per Sq. M.	Treatment	Mg. per Sq. M.
		Lead Deposited			
1	455	5	388	6	205
10	389	6	370	11	203
6	343	10	366	10	189
5	327	3	333	12	180
8	249	7	308	14	164
12	235	14	278	4	148
13	232	11	253	8	145
14	211	12	227	13	143
3	176	9	213	1	134
7	173	4	211	7	117
9	146	1	186	2	111
11	141	2	170	3	109
4	94	8	156	5	Lost
2	72	13	139	9	Lost
		LEAD REMAINING			
After spraying with Three 250-ml. Portions of Water		After Natural Rxposure to 7.25 inches of Rainfall			
10	317	10	260	6	148
5	278	5	203	12	143
6	271	6	175	11	131
13	222	7	160	14	130
14	209	11	148	7	111
7	160	12	128	10	89
3	149	14	125	2	89
11	124	3	84	1	84
8	105	2	82	8	65
9	104	9	80	3	57
12	82	13	76	4	36
4	79	4	76	5	Lost
1	62	8	66	9	Lost
2	32	1	43	13	Lost

The figures for lead on leaves and fruits in table 2 fail to show a close correspondence. Results secured from analyses of leaves can scarcely be used to reveal lead levels on the fruits. There was a better correspondence between leaves and plates.

DISCUSSION.—Hensill & Hoskins (1935) explained the selection of a beeswax surface for laboratory investigations of spray deposits in the following statement: "Since the surfaces of fruit and leaves are extremely varied and change with age, it seemed advisable to use an artificial surface which was of constant properties and easily reproducible." The authors have stated (Frear & Worthley, 1937, 1940), and Fahey & Rusk (1939) have developed more fully the point that under field conditions rainfall may be only a minor factor involved in the weathering away of lead arsenate spray deposits. The latter authors have considered in detail the conditions that should surround the selection of apple fruit samples for the chemical determination of spray residues.

In the present study the sprays were applied to clean *Pyralin* plates. The leaf and fruit surfaces, however, already bore some spray material. The possibility that both deposit and retention of spray mixtures may be different on *Pyralin* plates that already bear a spray film is the subject of further study. It is proposed also to compare deposits produced by the laboratory spraying apparatus described with those obtained through the use of a positive displacement pump and orchard type spray nozzle.

One source of error inherent in the attempt to reduce the fruit figures to amounts per standard unit of surface can be minimized only by the replication of treatment and sampling on several trees. This error arises first from the fact that in taking the fruit samples the assumption must be made that the apples taken on a given date were of the same size at the date of spraying (and consequently received about the same amount of spray material) as their neighbors selected for analysis immediately after the application. A second contributing factor is the error caused by differences in the exposure of different fruits to the spray application, and later to rainfall, drip from higher branches, wind action, and mechanical brushing. Doubtless other sources of error help to increase the difficulties involved in comparing laboratory and field spraying, especially where expanding plant surfaces are involved.

CONCLUSIONS.—The present laboratory study has revealed certain interesting relationships in the deposition and retention of lead from various spray mixtures. Certain of these were substantiated by the parallel field experiments. Further study is necessary to develop methods by which laboratory investigations of spray deposition and retention on artificial surfaces may safely be used to forecast field results with the same spray mixtures applied to various plant surfaces.

It is recognized that certain fundamental factors affecting the deposition and retention of spray materials can best be investigated through laboratory procedures that involve the use of a standard, artificial surface. However, until it can be shown that such a method gives results that correlate well with those secured in field applications to the plant surfaces in question, it should be used with caution as denoting the effect of adjuvant materials on deposition and retention of lead arsenate in field applications.—11-23-42.

LITERATURE CITED

Ben-Amotz, Y., and W. M. Hoskins. 1937. Factors concerned in the deposit of sprays. III. Effects of wetting and emulsifying powers of spreaders. JOUR. ECON. ENT. **30**(6): 879–86.

Dawsey, Lynn H., A. W. Cressman, and J. Hiley. 1937. The relative quantities of oil deposited upon paraffin-coated plates and upon plant foliage by oil sprays. Jour. Agr. Res. **54**(5): 387–98.

Fahey, Jack E., and Harold W. Rusk. 1939. A field method for the chemical evaluation of spray deposits resulting from the application of insecticides for control of the codling moth. U.S.D.A. Bur. Ent. and Plant Quar. **E-491.**

McCallan, S. E. A., and Frank Wilcoxon. 1940. An analysis of factors causing variation in spore germination tests of fungicides II. Methods of spraying. Contrib. Boyce Thompson Inst. **11**(4): 309–24.

Frear, D. E. H., and D. E. Haley, 1934. A simplified method for the rapid determination of lead residues on apples. Pa. Agr. Expt. Sta. Bul. **304.**

Frear, Donald E. H., and H. N. Worthley. 1937. Deposition and retention of sprays on apples. Pa. Agr. Expt. Sta. Bul. **344.**

Frear, Donald E. H., and Harlan N. Worthley. 1940. Deposition and retention of sprays on apples. II. Pa. Agr. Expt. Sta. Bul. **400.**

Hensill, G. S., and W. M. Hoskins. 1935. Factors concerned in the deposit of sprays. I. The effect of different concentrations of wetting agents. JOUR. ECON. ENT. **28**(6): 942–50.

Horsfall, J. G., J. W. Heuberger, E. G. Sharvelle, and J. M. Hamilton. 1940. A design for laboratory assay of fungicides. Phytopath. **30**(7): 545–63.

Hoskins, W. M., and E. L. Wampler, 1936. Factors concerned in the deposit of sprays. II. Effect of electrostatic charge upon the deposit of lead arsenate. JOUR. ECON. ENT. **29**(1): 134–43.

Reprinted July 10, 1942 from
JOURNAL OF ECONOMIC ENTOMOLOGY 35 (2) :205

Reprinted from JOURNAL OF DAIRY SCIENCE, July, 1942, Vol. XXV, No. 7, pages 571–583.

CAROTENE IN CALF NUTRITION[1]

H. A. KEENER AND S. I. BECHDEL
Department of Dairy Husbandry

N. B. GUERRANT
Department of Agricultural and Biological Chemistry

AND

W. T. S. THORP
Department of Animal Husbandry,
The Pennsylvania State College

The indispensable nature of vitamin A in the ration of dairy calves was shown by Jones, Eckles and Palmer (8) as early as 1926. Bechdel, Eckles and Palmer (1) reported similar findings the same year. Hart and Guilbert (7) found that a vitamin A deficiency developed in cattle under range conditions during unusually long seasons of dry feeding. Guilbert and Hart (4) reported that 26–33 micrograms of carotene from alfalfa per day per kilogram of body weight was sufficient to prevent or cure vitamin A deficiency in cattle. Guilbert, Miller and Hughes (5) also reported the minimum carotene requirement for cattle, sheep and swine to be between 25 and 30 micrograms per day per kilogram of body weight. The vitamin A requirement was found to be 6–8 micrograms daily per kilogram of body weight. However, data obtained by Halverson, Hostetler, Foster and Sherwood (6) indicated that the minimum daily carotene requirement of cattle was 43 to 55 I. U. per kilogram of body weight.

Moore (10) found that a carotene intake of about 16 micrograms per pound of body weight per day was sufficient to maintain plasma carotene at 0.2 microgram per milliliter, to prevent nyctalopia and to maintain a fair state of general health. When plasma carotene values fell below 0.13 microgram per milliliter, nyctalopia and papillary edema were found to follow. Converse and Meigs (2) concluded that because of the greater susceptibility of calves to various calf ailments during the first 3–4 months of life than at older ages they need a more generous allowance of vitamin A than at older ages. These workers concluded that the California standard (4) is less than one-third of the optimum for calves between 3 and 180 days of age. Ward, Bechdel and Guerrant (12, 13) found that 11 micrograms of carotene from a carotene concentrate per pound of body weight per day prevented vitamin A deficiency in Holstein calves. This requirement was also met by 14

Received for publication March 5, 1942.

[1] Authorized for publication on March 4, 1942, as paper No. 1089 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The data presented in this paper are taken from a thesis submitted by Harry A. Keener to the Graduate School of The Pennsylvania State College in partial fulfillment of requirements for the degree of Doctor of Philosophy.

micrograms of carotene from alfalfa hay or by 33 micrograms of carotene from alfalfa molasses silage. It was also observed that the carotene requirements of some calves appeared to be slightly higher in cold than in mild or moderate weather. The present experiments are a continuation of the previous studies, using certain improvements in experimental technic.

EXPERIMENTAL

Thirty-four calves representing the Guernsey and Holstein breeds were used in these experiments to determine the minimum amount of carotene that would prevent vitamin A deficiency symptoms in dairy calves from the time of birth to 6–8 months of age. The experimental results obtained with 24 of these experimental subjects are reported at this time. All calves were removed from their dams at 2 to 4 days of age and were placed on reconstituted skimmilk at 4 to 7 days of age, except the first 6 calves which were kept on whole milk until 4 weeks of age. A ration composed of wheat bran, rolled oats, ground barley, linseed oil meal, soybean oil meal, molasses, steamed bone meal, salt and irradiated yeast was fed according to appetite. The above ration contained 13.0 per cent digestible crude protein and 72.2 per cent total digestible nutrients. Dried beet pulp, as the sole roughage, was fed according to appetite.

Calves were maintained in individual stalls in a well ventilated and well lighted calf barn. Although artificial heat was used in the barn at times, the temperature therein was not maintained as constant as was hoped, particularly during cold and windy weather. Sawdust was used as the bedding.

Alfalfa leaf meal of high quality was used as the principal source of carotene. It was weighed into gelatin capsules and fed with a balling gun. While the plan of the experiment was to keep each calf on a definite daily carotene intake (per pound of body weight), small variations in daily dosages did occur due to changes in the carotene content of the alfalfa leaf meal and to slight variations in body weight. Carotene assays on the alfalfa leaf meal were made by both the biological and the colorimetric methods.

The experimental animals were weighed at weekly intervals and the carotene intake adjusted according to changes in weight. The eyes of the calves were examined weekly by means of an opthalmoscope according to the method of Moore (9). A complete record was made of all other visible conditions that appeared to have any relation to a deficiency of carotene in the diet.

Blood carotene determinations were made every two weeks by a method developed for this work. This consisted in precipitating whole blood with methanol, extracting with petroleum ether, saponifying and then determining the carotene concentration by means of a photoelectric colorimeter. During the last 10 months of the experiment an aliquot from each petroleum ether extract was evaporated to dryness and the residue was taken up in isopropyl alcohol. Vitamin A readings were made at 328 millimicrons by means of an electronic photometer. While these data, in all probability, are only relative, they are of value in comparative studies.

The calves were slaughtered when removed from the experiment. A careful examination was made of the various organs for gross pathological conditions. Microscopic studies were conducted on tissues from the various organs. A summary of these observations has been published elsewhere (11).

DATA

Table 1 gives a summary of the experimental set-up employed and the results obtained with the different experimental animals. The season of the year during which each calf was on experiment should be considered in studying this table.

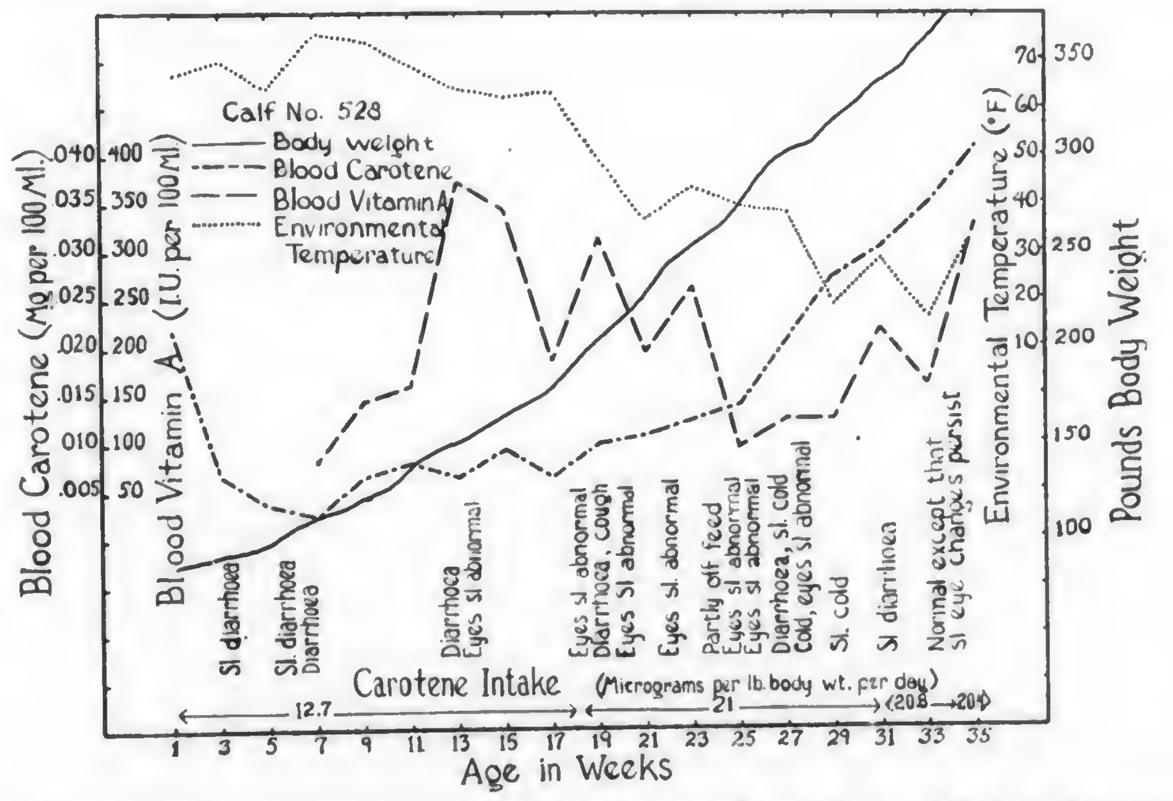


FIG. 1. Illustrating the relationship that was found to exist between environmental temperature, body weight changes, blood carotene, blood vitamin A level, and the general condition of calf No. 528.

Figures 1 and 2 illustrate with individual calves the relationship that was found to exist between environmental temperature, the body weight changes, the blood carotene level, the blood vitamin A level and the condition of the calf. Figure 3 illustrates further the relationship found between the carotene and the vitamin A levels in the blood and the environmental temperature, the carotene and vitamin A values being the averages from six animals.

DISCUSSION OF RESULTS

Examination of the eye by means of an opthalmoscope appeared to be the most sensitive method of detecting the effects of vitamin A deficiency. The chief defect of this method was due to changes produced in the eye by vitamin A deficiency which frequently persisted for weeks or even months after the carotene intake had been increased to an apparently adequate level.

The blood carotene and vitamin A levels seemed to indicate rather reliably the adequacy of the carotene intake, although here again individual variations were observed. The vitamin A level appeared to be a better indicator of an adequate intake of vitamin A than did the blood carotene level. This is what one might expect since it is vitamin A *per se* that is used by the body and not carotene. However, it appeared that when blood carotene fell below .0175 milligram per 100 milliliters of whole blood in Holstein calves, vitamin A deficiency symptoms were frequently found. Above this figure vitamin A deficiency symptoms were rarely observed. Such a value for Guernsey calves appeared to be somewhat higher. It seems undesirable at this time to attempt to set a minimum value for blood vitamin A because the values determined in these experiments may be only relative. However, Davis and Madsen (3) have set 25 micrograms of carotene and 16 micrograms of vitamin A per 100 milliliters of plasma as the critical level.

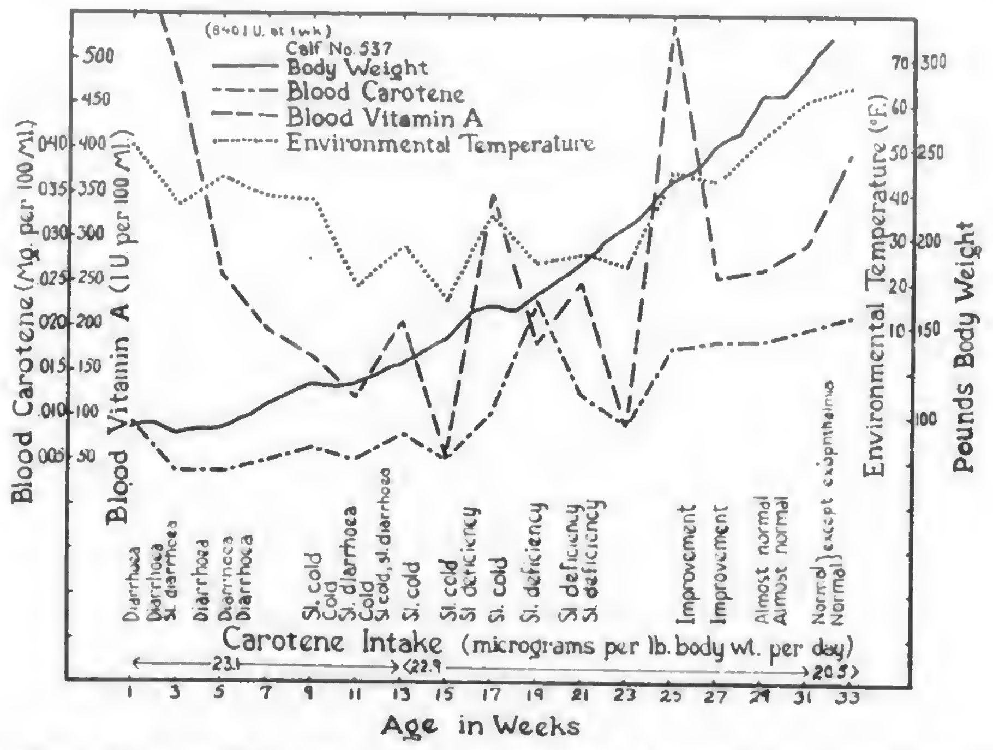


FIG. 2. Illustrating the relationship that was found to exist between environmental temperature, body weight changes, blood carotene, blood vitamin A level, and the general condition of calf No. 537.

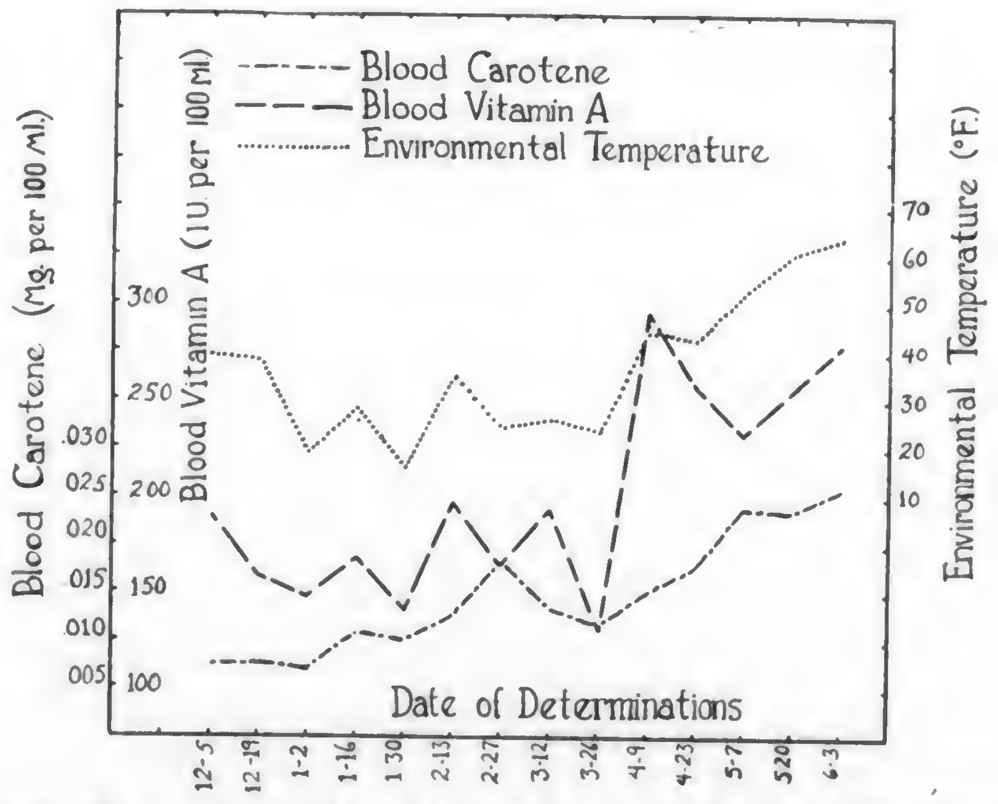


FIG. 3. Illustrates the relationship found to exist between environmental temperature, blood carotene and blood vitamin A (average values of six representative calves).

The observed effects of vitamin A deficiency in calves were similar to those previously reported by other workers. These included dilated pupils, bleached tapetum lucidum, exophthalmia, papilledema, roughness of coat, poor flesh, slowness of growth, muscular incoordination, humped back, diarrhea, colds, pneumonia, blindness and death. The gross and microscopic pathology observed at autopsy included a cystic condition of the pituitary, a constriction of the optic foramen and various degrees of histopathological changes in the tissues of the intestines, liver, kidney and testicle.

An observation which appears to be of considerable importance is the relationship which appeared to exist between the vitamin A content of the blood and the environmental temperature. This is illustrated by all three figures (1–3 inclusive). This relationship indicates that either vitamin A is required in greater amounts during cold weather or that more of the vitamin is oxidized by the increased metabolism required to maintain body temperature. Respiratory disturbances and diarrhea appeared to be more prevalent during periods of low blood vitamin A thus adding another complicating factor to the situation. The fact that on the higher levels of carotene intake these conditions were observed only rarely, appears to eliminate them as the cause of the decreased blood vitamin A. The logical assumption is that these conditions were brought about in the calves on the lower levels of carotene intake as a result of the low concentration of blood vitamin A or by the same conditions which produced the low blood vitamin A.

The effects of vitamin A deficiency on the intestinal mucosa would seem to act toward producing a sort of vicious circle. Under these conditions, vitamin A deficiency tends to produce an enteritis which results in a diarrhea and probably in a decreased absorption of carotene. This would tend to make the deficiency more marked and thus produce a more severe enteritis. The importance of adequate dietary vitamin A, in this respect, can be readily appreciated. Colds followed by acute bronchial pneumonia might also tend to act in a similar manner. The vitamin A deficiency probably tends to cause a decrease in the resistance of the upper respiratory tract against infection. The infection produced then tends to increase heat production by means of a fever, thus probably bringing about an increased destruction of vitamin A and a greater vitamin A deficiency. Although most of this is still theory, it may offer explanations for some of the phenomena observed.

Another observation which may be of considerable importance was the greater amount of vitamin A in relation to carotene in the blood of young calves as compared to that of older calves. This observation would indicate that nature has made it so that the young animal converts carotene to the essential vitamin A either more efficiently or more rapidly as a means of giving it increased protection during this critical period of life. It is possible that the difference in the ability to convert carotene to vitamin A may be responsible for the greater ease with which Holstein calves are raised than is the case with Guernseys. This factor may function both before and after birth.

Actual observations bear out the difference in carotene requirement indicated by blood vitamin A levels at different environmental temperatures. Calves which received around 12 micrograms of carotene per pound of body weight per day during warm weather appeared to get just about enough to prevent vitamin A deficiency symptoms. However, calves that received 20 to 23 micrograms of carotene per pound of body weight per day during the winter months have been observed to show deficiency symptoms during or immediately following periods of unusually cold weather. The effects of weather changes were very evident in the eyes of the affected calves. Further effects of temperature changes were indicated by the vitamin A and carotene values obtained on the livers.

The gross and microscopic studies made on the organs and tissues support the observation just noted. The extent and severity of these changes were almost without exception roughly proportional to the severity of the external symptoms of vitamin A deficiency observed, particularly after an animal had shown deficiency symptoms for some time. It is important to note that definite histopathological effects of vitamin A deficiency were found in animals which had been considered to be getting the minimum carotene intake. Practically all such animals would be considered normal by the average dairyman. This brings up the important fact that such cases as these on the borderline of deficiency are probably very widespread, particularly in herds in regions where the roughage is usually of poor quality. It is highly probable that such effects might result in the production of cows that would lack in vigor, in milk producing ability, and in reproductive efficiency, without the real cause being suspected. It is possible that the most detrimental effect of previous vitamin A deficiency in dairy cattle lies in such cases.

CONCLUSIONS

1. The minimum carotene requirement of dairy calves maintained at an environmental temperature of 50 to 70° F. was found to be approximately 12 micrograms per pound of body weight per day.

2. The minimum carotene requirement for growth and well-being of dairy calves appears to depend upon environmental temperature. During severe winter weather the minimum requirement may be more than twice as great as during warm weather. This increased requirement is substantiated by the observed lowering of blood carotene and blood vitamin A during cold weather.

3. Respiratory and bowel disturbances were more prevalent during periods of low blood vitamin A than when the store of blood vitamin A was more abundant.

TABLE 1

Summary of the experimental set-up employed and the results obtained with the individual calves

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E17	M	G	7–28–39	3.7 at 9–28 weeks of age	Papilla—atrophied Pituitary—cystic Liver—scarred Kidney—grayish yellow foci Intestine—marked thickening of mucosa Blindness	Kidney—degeneration of tubules Liver—degeneration and necrosis Intestine—inflammatory changes	Definitely deficient after 7 weeks of age
E18	M	G	8–13–39	11.8 at 11–15 weeks of age 12.7 at 16–29 weeks of age	Normal except diarrhea	Kidney—slight degeneration of some of tubular epithelium	Deficient 5–14 weeks, about on borderline thereafter
E19	F	G	7–27–39	4.7 at 10–15 weeks of age 5.1 at 16–28 weeks of age	Papilledema Lungs—consolidated in small area Heart valves—small cysts Intestinal epithelium—hyperplastic Stomach—hemorrhagic Gall bladder—walls thickened Nearly blind	Kidney—degeneration Intestine—inflammatory changes Liver—degeneration and necrosis	Deficient after 8 weeks of age
E20	M	G + H	8–14–39	10.7 at 10–15 weeks of age 11.4 at 16–29 weeks of age	None	Kidney—slight degeneration Liver—several foci of necrosis	Deficient at 7 weeks. Gradually recovered. On borderline after 13 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E21	M	G	8–16–39	5.9 at 12–17 weeks of age 6.4 at 18–32 weeks of age	Pituitary—slightly cystic Optic nerve—moderately constricted Liver—slight scarring of parenchymatous tissue Kidney—small grayish white areas on sectioning Intestinal mucosa—mild hyperplasia Papilledema	Kidney—marked degeneration of tubules Testicle—marked degeneration of germinal epithelium Intestine—extensive hyperplastic enteritis	Deficient after 8 weeks of age
E23	F	G	8–16–39	7.1 at 10–15 weeks of age 7.6 at 16–30 weeks of age	Kidney—small grayish white areas on sectioning Intestine—moderate enteritis of subacute nature Papilledema	Kidney—slight glomerular nephritis Intestine—moderate inflammatory changes	Deficient after 7 weeks of age—slight to moderate changes
510	F	H	10–24–39	7.1 at 5–10 weeks of age 7.6 at 11–34 weeks of age	Pituitary—cystic Intestine—marked hyperplasia Optic nerve—constricted Papilledema Kidney—small grayish white foci on surface and cut section	Kidney—early changes Liver—slight degeneration Intestine—moderate inflammatory changes	Deficient after 10 weeks of age. Improved from 29 weeks until slaughtered at 34 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
513	M	H	9– 7–39	9.5 at 3–8 weeks of age 10.0 at 9–26 weeks of age	Papilledema Intestinal mucosa—very thickened and severe diarrhea Lungs—slight congestion	(Not examined)	Deficient after 9 weeks of age
514	M	H	10–25–39	10.7 at 3–8 weeks of age 11.4 at 9–32 weeks of age	Papilledema	Kidney—moderate changes Liver—slight changes Intestine—slight enteritis	Borderline of deficiency 17–25 weeks. Deficient thereafter
516	M	H	10–20–39	4.7 at 2–7 weeks of age 5.1 at 8–31 weeks of age	Pituitary—cystic Optic nerve—markedly constricted Papilledema Small intestine—very hyperplastic Almost blind	Kidney—tubular degeneration and fibrosis Liver—fatty degeneration Small intestine—hyperplastic enteritis Testicle—slight degeneration	Deficient after 4 weeks of age
521	M	H	12–15–39	8.3 at 3–6 weeks of age 8.9 at 7–30 weeks of age 14.7 at 31–38 weeks of age	Pituitary—cystic Intestine—mild enteritis above iliocecal valve Papilledema	Kidney—Early degenerative and inflammatory changes Liver—marked changes Intestine—marked changes	Near borderline 14–26 weeks. Deficient thereafter

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
523	M	H	12– 3–39	6.4 at 2–25 weeks of age 10.5 at 26–32 weeks of age	Papilledema Pituitary—cystic Lungs—anterior lobes consolidated and abscessed Kidney—Small foci on cortical surface	Kidney—early degenerative and inflammatory changes Intestine—marked changes Liver—marked changes	Deficient after 12 weeks. Gradually became worse
524	M	H	1– 9–40	11.4 at 2–25 weeks of age 18.9 at 26–37 weeks of age	None	Kidney—some degeneration and cellular infiltration	On or just below borderline after 26 weeks of age (cold weather)
528	M	H	2–13–40	12.7 at 2–18 weeks of age 21.0 at 19–35 weeks of age	None	Kidney—slight degeneration and slight cellular infiltration Testes—considerable degeneration	On borderline 25–33 weeks (cold weather). Appeared O.K. when killed
529	M	H	2–12–40	16.5 at 2–16 weeks of age 27.3 at 17–33 weeks of age	None	Kidney—early changes with a few areas of fibrosis and hemorrhages in tubules	Near borderline during last several weeks (cold weather)
533	M	H	6– 4–40	10.2 at 2–6 weeks of age 16.8 at 7–21 weeks of age 18.4 at 22 weeks of age and decreased gradually to 16.8 at 39 weeks	Slight papilledema remained	Intestine—a moderate enteritis	Slight deficiency 12–33 weeks (cold weather). Appeared sufficient 34–39 weeks

---

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
534	M	H	5–24–40	12.7 at 2–5 weeks of age 21.0 at 6–18 weeks of age but then decreased gradually to 18.8 at 36 weeks	None	(Not examined)	Slight deficiency 24–29 weeks (cold weather). Appeared normal thereafter
537	M	H	6– 4–40	23.1 at 2–13 weeks of age and then gradually decreased to 20.5 at 33 weeks	Pituitary—slighlty cystic Eyes—exophthalmia and slight papilledema Lungs—some consolidation Heart—cystic ventricular valves	Intestine—moderate enteritis	Slightly below borderline 16–24 weeks (cold weather). Intake appeared sufficient after 25 weeks of age
538	M	H	6–10–40	23.1 at 2–14 weeks of age 18.5 at 15 weeks of age and decreased gradually to 16.8 at 32 weeks of age	Slight eye changes remain	Intestine—mild enteritis Liver—slight degeneration in few areas	Slightly deficient 17–27 weeks (cold weather). Intake appeared adequate at end
E24	M	G	6–10–40	18.9 at 3–13 weeks of age then decreased gradually to 16.8 at 34 weeks	None	(Not examined)	On borderline of deficiency 9–25 weeks (cold weather). Sufficient 27–34 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E25	M	G	6–10–40	21.0 at 2–12 weeks of age then decreased gradually to 18.6 at 33 weeks	None	(Not examined)	Slight deficiency 17–26 weeks (cold weather). Sufficient 27–33 weeks
E30	M	G	2– 2–40	23.1 at 2–15 weeks of age	None	Kidney—some degeneration and some cellular infiltration	On borderline of deficiency
548	M	H	6–27–40	16.3 at 2 weeks of age gradually decreased to 14.9 at 20 weeks of age, then 3 weeks with none	Papilledema—developed during last 3 weeks Left eye—almost blind at end of experiment	Kidney—early changes Intestine—slight enteritis	Just about enough except slight deficiency 10–16 weeks (cold weather). Deficient at end
551	M	H	6–27–40	6.1 at 2 weeks of age decreased gradually to 5.6 at 19 weeks of age. None last 3 weeks	Papilledema Exophthalmia Blindness	Kidney—moderate changes including some degeneration of tubules and cellular infiltration	Deficient after 10 weeks and gradually became worse

4. The severity of the gross and microscopic pathology agreed very well with the degree of deficiency observed before the calves were slaughtered.

5. Histopathological studies reveal that calves receiving a carotene intake of less than 27 micrograms per pound of body weight per day may not be fully protected when subjected to average winter conditions.

6. It is highly probable that such histopathological changes exist in calves raised in regions where roughage of poor quality is fed and that these conditions may affect the productive and reproductive efficiency of the dairy cattle of such regions.

REFERENCES

(1) BECHDEL, S. I., ECKLES, C. H., AND PALMER, L. S. The Vitamin B Requirement of the Calf. JOUR. DAIRY SCI., 9: 409–438. 1926.

(2) CONVERSE, H. T., AND MEIGS, E. B. The Vitamin A Requirements for Normal Growth in Young Dairy Cattle. Amer. Soc. Anim. Prod. Proc., 67–72. 1939.

(3) DAVIS, R. E., AND MADSEN, L. L. Carotene and Vitamin A in Cattle Blood Plasma with Observations on Reproductive Performance at Restricted Levels of Carotene Intake. Jour. Nutr., 21: 135–146. 1941.

(4) GUILBERT, H. R., AND HART, G. H. Minimum Vitamin A Requirements with Particular Reference to Cattle. Jour. Nutr., 10: 409–427. 1935.

(5) GUILBERT, H. R., MILLER, R. F., AND HUGHES, E. H. The Minimum Vitamin A and Carotene Requirement of Cattle, Sheep and Swine. Jour. Nutr., 13: 543–564. 1937.

(6) HALVERSON, J. O., HOSTETLER, E. H., FOSTER, J. E., AND SHERWOOD, F. W. Soybean Hay, Alfalfa Hay and Yellow Corn as Vitamin A Supplements in Cottonseed Meal Rations for Growing Cattle. Jour. Nutr., 18: 285–296. 1939.

(7) HART, G. H., AND GUILBERT, H. R. Vitamin A Deficiency as Related to Reproduction in Range Cattle. Calif. Agr. Expt. Sta. Bul. 560: 1–30. 1933.

(8) JONES, I. R., ECKLES, C. H., AND PALMER, L. S. The Role of Vitamin A in the Nutrition of Calves. JOUR. DAIRY SCI., 9: 119–139. 1926.

(9) MOORE, L. A. Technique Used in Studying Vitamin A Requirements of Dairy Cattle. JOUR. DAIRY SCI., 20: 427. 1937.

(10) MOORE, L. A. Carotene Intake, Level of Blood Plasma Carotene, and the Development of Papillary Edema and Nyctalopia in Calves. JOUR. DAIRY SCI., 22: 803–812. 1939.

(11) THORP, W. T. S., KEENER, H. A., BECHDEL, S. I., AND GUERRANT, N. B. Amer. Jour. Vet. Res., pp. 22–26. Jan., 1942.

(12) WARD, R. E., BECHDEL, S. I., AND GUERRANT, N. B. The Carotene Requirement of Dairy Calves. JOUR. DAIRY SCI., 21: A168–A169. 1938.

(13) WARD, R. E., BECHDEL, S. I., AND GUERRANT, N. B. Carotene and Vitamin A in Nutrition of Growing Dairy Cattle. JOUR. DAIRY SCI., 23: 115–124. 1940.

Reprint from American Journal of Veterinary Research, Vol. III, No. 9, October 1942, pp. 329-335.

A Study of the Administration of Sulfanilamide to the Normal Dairy Cow

ISADORE ZIPKIN, Ph.D., ARTHUR K. ANDERSON, Ph.D., and W. T. S. THORP, D.V.M.
State College, Pa.

WITHIN THE LAST few years, sulfanilamide has been given more attention than perhaps any other drug in the treatment of bovine mastitis.[1, 2, 9, 14, 15] Only a few clinical studies of the administration of sulfanilamide to the normal dairy cow have appeared in the literature. Perhaps the first report dealing with the elimination of sulfanilamide in the milk was made by Schlotthauer in 1938.[16] Later, Gildow, *et al.*,[5] made one of the most intensive studies of the absorption of sulfanilamide by determining its concentration in the blood and milk as affected by different levels of dosage.

Only a study of the concentration of free sulfanilamide in the blood and milk has appeared in the literature. Marshall,[11] however, has shown that various animals may conjugate sulfanilamide as the acetyl derivative. The acetylated compound in most instances has been shown to be inactive. Hence, it was felt that the determination of conjugated sulfanilamide in the blood and milk would show a double significance. It would give a more accurate picture of the fate of ingested sulfanilamide and, furthermore, might explain the inefficiency of sulfanilamide in many cases of mastitis.

In view of the well-known toxic effects of sulfanilamide on the bone marrow of human subjects,[3, 7, 8] it seemed obvious that a study of the hemoglobin and the cellular constituents of the blood of dairy cows undergoing sulfanilamide administration would provide an index of the toxicity of the drug.

The present investigation deals with the effects of different doses of sulfanilamide on the normal dairy cow. This study was approached from two viewpoints: (1) a study of the free and total sulfanilamide content of the blood and milk, and (2) its effect on the blood picture; namely, on the erythrocyte, leucocyte, and differential counts and the hemoglobin content. The term total sulfanilamide includes free sulfanilamide as well as that present in the conjugated form. During the course of the investigation, a careful record was made of the effect of sulfanilamide on the daily milk production.

EXPERIMENTS

In this study, four Holstein-Friesian cows of known weight, free from mastitis and normal in all other respects, were chosen from the dairy herd of the Department of Dairy Husbandry, Pennsylvania State College.

Blood samples were drawn from the jugular vein daily after the 2:00 p.m. milking period. It has been found that bleeding just before milking interferes with milk production. The cows were milked each day at 6:00 a.m., 2:00 p.m., and 10:00 p.m. One one-hundredth aliquots were taken at each milking period and pooled, and a sample was taken from the pooled aliquots for analysis of free and total sulfanilamide.

During the first control period, neither milk nor blood was analyzed for sulfanilamide, since previous tests had indicated that normal blood and milk give no color with the reagents used in the determination of sulfanilamide. During the first experimental period, the sulfanilamide

---

Authorized for publication on July 7, 1941, as paper No. 1041 in the journal series of the Pennsylvania Agricultural Experiment Station.

From the Departments of Agricultural and Biochemical Chemistry and Animal Husbandry of Pennsylvania State College.

was administered in half-ounce gelatin capsules with a balling gun in 2 equal doses at 12-hour intervals, at 9:00 a.m. and at 9:00 p.m. Since the second level of dosage necessitated the use of a large number of capsules, it was found advisable, during the second experimental period, to administer the sulfanilamide by drenching. The administration of sulfanilamide was discontinued during the fourth period of the investigation, designated as the final control period. Milk samples were taken daily until both free and conjugated sulfanilamide could no longer be detected in them. Blood samples also were drawn daily until the hemoglobin values and the cell counts had returned to normal. At the conclusion of the investigation, the animals were weighed to determine the loss in weight following the use of sulfanilamide.

Free and total sulfanilamide were determined in a laked blood filtrate, according to the method of Marshall.[10] With a slight modification, the method was adapted for the determination of the above in milk; water was used as the diluent instead of saponin solution. In all other respects, the procedure was identical for both blood and milk. The procedure for determining both free and total sulfanilamide is the same except that in the latter, an aliquot of the blood or milk filtrate is first hydrolyzed. The results for both are given in milligrams per 100 cc. of sulfanilamide.

Erythrocyte and leucocyte counts were made in duplicate in the usual manner. Hemoglobin was determined by the acid hematin method of Newcomer,[13] using a standard color plate. All colorimetric readings were made in daylight. For the differential count, Wright's stain was employed, a phosphate buffer of pH 7.0 being used as the diluent in place of distilled water. For each determination, 200 cells were counted, 100 from each of two duplicate slides.

A summary of the plan of the investigation is given in table I. It was necessary to modify the original plan to prevent overdosage, as explained in the following section.

PRESENTATION OF DATA

Four cows, 184, 186, 204, and 239, were chosen for this investigation. Since the results obtained on the different cows were in reasonable agreement, only the data for two animals, cows 184 and 204, are presented.

TABLE I.—Showing the Plan of the Investigation

PERIOD	DATE	DOSAGE PER DAY PER 100 LB. BODY WEIGHT	DETERMINATIONS MADE ON BLOOD	DETERMINATIONS MADE ON MILK
Initial control	July 10-21, 1939	None	Erythrocyte count Leucocyte count Differential count Hemoglobin	None
First experimental	July 24-31, 1939	5 Gm.	Erythrocyte count Leucocyte count Differential count Hemoglobin Free sulfanilamide Total sulfanilamide	Free sulfanilamide Total sulfanilamide
Second experimental	July 31-Aug. 7, 1939	10 Gm.		
Final control	Aug. 7-18, 1939	None		

During the course of this investigation, it became evident that the original plan of the investigation as outlined in table I could not be followed, since the animals reacted unfavorably to the drug. In every case, the cows went off feed at some period during the study. The administration of sulfanilamide was then discontinued for a period and instituted again as indicated on the graphs in fig. 1. Symptoms which necessitated discontinuance of the drug were a slightly inflamed udder, diarrhea, and a general unfavorable systemic appearance. The cows did not ruminate, were listless, and seemed to be in a state of general malaise. It is possible that the diarrhea was due in part to the ration, since freshly cut soybean forage and Sudan grass were fed during the period when the diarrhea was noticed. Refusal of the feed for more than one day was considered sufficient cause to discontinue the administration of sulfanilamide.

A.—Concentration of Total, Conjugated, and Free Sulfanilamide in the Blood and Milk.—The concentration of total, conjugated, and free sulfanilamide in the blood and milk following administration of the drug is presented in fig. 1. The concentration of the conjugated or acetylated sulfanilamide was calculated by taking the difference between the values representing total and free sulfanilamide. The lower level of dosage, 5 Gm. per 100 pounds of body weight daily, is referred to as the first dosage, and the higher level is called the second dosage. The legend for the curves in parts *A* and *C* of fig. 1 also refers to parts *B* and *D*, for the same animals respectively.

On the low level of dosage, the maximum concentration of total sulfanilamide in the blood of cow 184 was found to be 6.5 mg. per 100 cc., whereas in the milk of the same animal, a concentration of 12.7 mg. per 100 cc. was reached. At first glance, it seems strange that the concentration of total sulfanilamide in the milk should exceed that in the blood. An examination of the partition of total sulfanilamide, however, shows that about twice as much of the conjugated form is present in the milk as in the blood. In the blood, a maximum concentration of 2.4 mg. per 100 cc. of conjugated sulfanilamide was found, representing 37 per cent of the total sulfanilamide, while in the milk, 8.8 mg. per 100 cc. were conjugated, representing 69 per cent of the total sulfanilamide. On the same level of dosage, the free sulfanilamide distributed itself approximately equally in the blood and milk, 4.7 mg. per 100 cc. being the maximum found in the blood, and 4.2 mg. per 100 cc., the maximum found in the milk.

On the high level of dosage, however, it was surprising to note that sulfanilamide was present in the blood almost wholly in the free form. A maximum concentration of free sulfanilamide of 17.5 mg. per 100 cc. was reached in the blood of cow 184. Despite the fact that little conjugated sulfanilamide could be found in the blood on the high level of dosage, a large amount was present in the milk. A maximum concentration of total sulfanilamide in the milk of 27.4 mg. per 100 cc. was reached. For the same day, the milk showed a concentration of conjugated sulfanilamide of 15.4 mg. per 100 cc., representing 56 per cent of the total sulfanilamide. The free sulfanilamide was found to reach a concentration of 12.0 mg. per 100 cc.

It will be noted that, in general, the concentration of free sulfanilamide in the milk did not exceed that in the blood on either level of dosage. From this it would appear that the passage of the unconjugated compound from the blood into the milk could be accounted for on the basis of diffusion. However, when one examines the data on conjugated sulfanilamide, it is more difficult to explain how the concentration in the milk is higher on both levels of dosage than that in the blood. Obviously, diffusion is not the complete answer. It is still more difficult to explain why on the high level of dosage there is so little conjugated sulfanilamide in the blood, when the concentration in the milk is high.

It should be pointed out that in this study no attempt has been made to determine the nature of the conjugated compound. Marshall[11] has found that, in animals where conjugation occurs, the conjugated compound is the acetyl derivative, and we have assumed that this is the case in this study. However, it would seem possible that many compounds other than acetic acid in the blood and milk might combine with sulfanilamide. Marshall[11] has also stated that conjugation takes place mainly in the liver. The results obtained in this study might be interpreted by assuming that the mammary gland also has the ability to conjugate sulfanilamide. It would appear possible that the conjugated compound in the milk may not be the same as that in the blood. Since milk has quite a different composition from that of blood, it is possible that, in the milk, sulfanila-

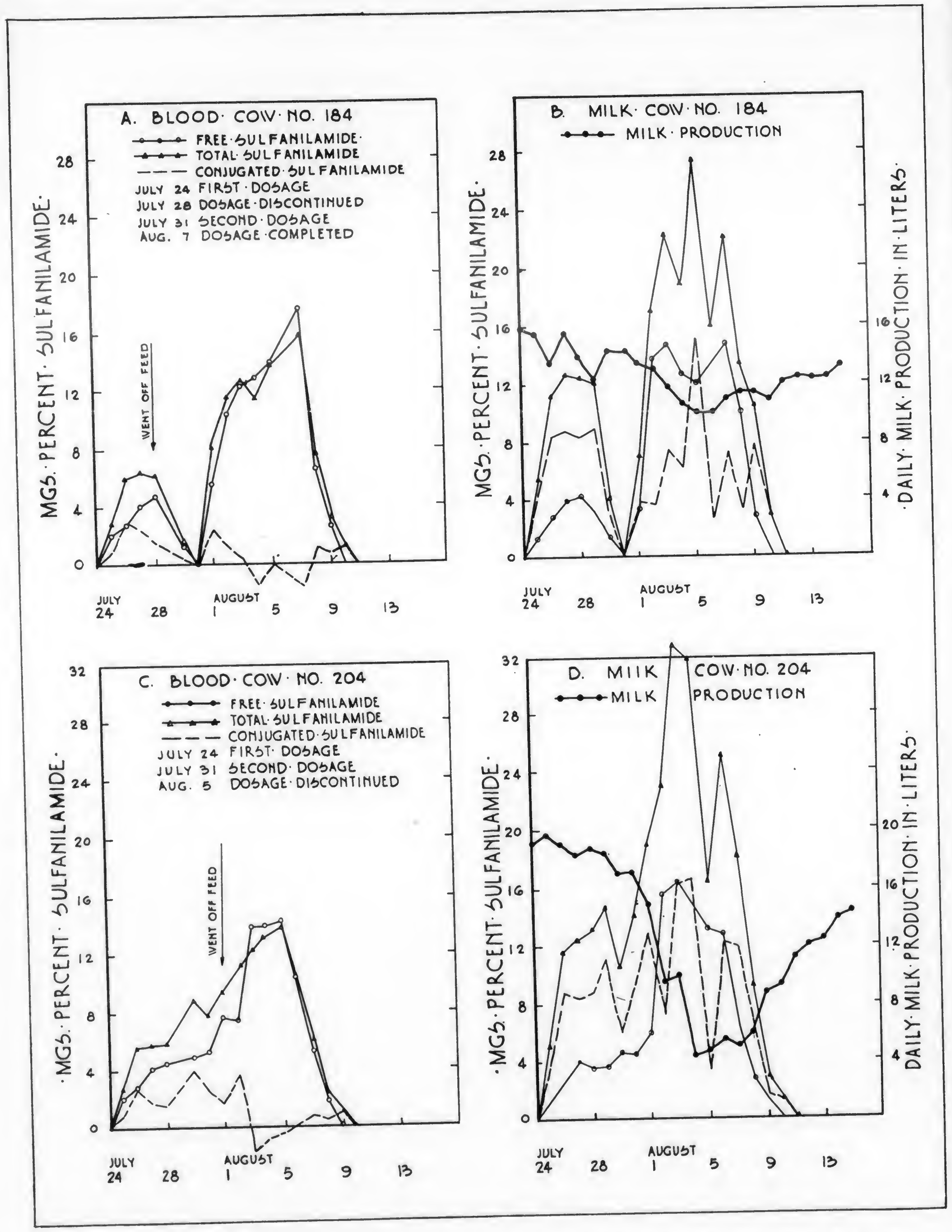


Fig. 1—The effect of sulfanilamide administration on the concentration of total, conjugated and free sulfanilamide in the blood and milk.

mide is conjugated with some constituent not found in the blood. An example of such a possibility would be the formation of a glucoside complex with lactose. If such compounds were formed, it would not be difficult to explain the high concentration of conjugated sulfanilamide in the milk.

The low concentration of conjugated sulfanilamide in the blood on the high level of dosage might be accounted for in two ways: (1) the concentration of free sulfanilamide in the blood on the high level of dosage may be sufficient to injure the liver tissue and thus interfere with its conjugating function, and (2) it is possible that with long continued administration, the compound in the blood which normally conjugates with it may be depleted. In that case it would be expected that no more conjugation would take place.

It would be interesting to attempt to isolate the conjugated derivatives from blood and milk and determine their nature. With this information, it should be possible to give a more satisfactory explanation of the high concentration of conjugated sulfanilamide in the milk.

With the advent of sulfanilamide, it was hoped that it would prove to be a valuable therapeutic agent in the treatment of mastitis. The high concentrations of total sulfanilamide found in the milk in this study would appear to favor the use and efficacy of the drug in this condition. It should be noted, however, that the greater proportion of sulfanilamide in the milk exists in the conjugated form, which has been shown to be therapeutically inactive. It has also been shown that free sulfanilamide tends to distribute itself equally in the blood and milk, in levels therapeutically effective in many cases of streptococcic infections. Since many cases of mastitis do not respond to sulfanilamide therapy, and since the concentration of the drug in both the blood and milk is sufficient for other therapeutic uses, it would appear that the organisms responsible for mastitis are not particularly sensitive to sulfanilamide.

Three days were required for the free sulfanilamide to disappear from the blood and milk of animal 184 following the last dose. An additional day was required for the conjugated compound to disappear completely from the blood and milk.

The daily milk production during the experimental and the final control periods is also presented in fig. 1*B* and 1*D*, to show the correlation between the milk flow and the concentration of sulfanilamide in the milk. As is particularly well illustrated in fig. 1*D*, the milk production varied inversely with the concentration of sulfanilamide in the milk. The milk flow returned to normal slowly, a maximum recovery of 83 per cent being observed in cow 184, 11 days after the final dose.

Similar results have been obtained for cow 204, except for the reduction in milk flow. A maximum reduction of 78 per cent was found in this animal, as compared with a 38 per cent reduction for animal 184. It should be remembered, however, that the same plan of dosage was not followed for both animals, as indicated in fig. 1*A* and 1*C*.

B.—Cellular Constituents of the Blood.—The effect of the administration of sulfanilamide on the cellular constituents of the blood, namely, the red and white cell count and the differential count, is presented in fig. 2. The hemoglobin value is also presented here to show its correlation with the erythrocyte count. To conserve space, only the data for cow 184 are presented. Normal values were determined for a period of 10 days preceding the experimental periods. The average value for the hemoglobin content and the cellular constituents of the blood is indicated in the graphs in fig. 2 by the straight lines designated "average normal." The administration of sulfanilamide is indicated by the arrows under the lymphocyte curve in the bottom graph of fig. 2 and applies to all the curves in the same figure. Since only the neutrophile and lymphocyte counts of the differential counts showed any significant variation from the normal, they alone are presented in fig. 2.

During the initial control period, an average hemoglobin value of 9.80 Gm. per 100 cc. of blood was obtained. On the low level of dosage, a slight increase in the hemoglobin was produced. On the high level of dosage, however, a decreasing trend was noted which reached a minimum of 7.89 Gm. per 100 cc., representing a decrease of 19 per cent from the normal value for animal 184.

A normal erythrocyte count of 6,700,000 per cubic millimeter of blood was obtained. During the experimental periods, the erythrocyte response closely paralleled the hemoglobin values as indicated in fig. 2.

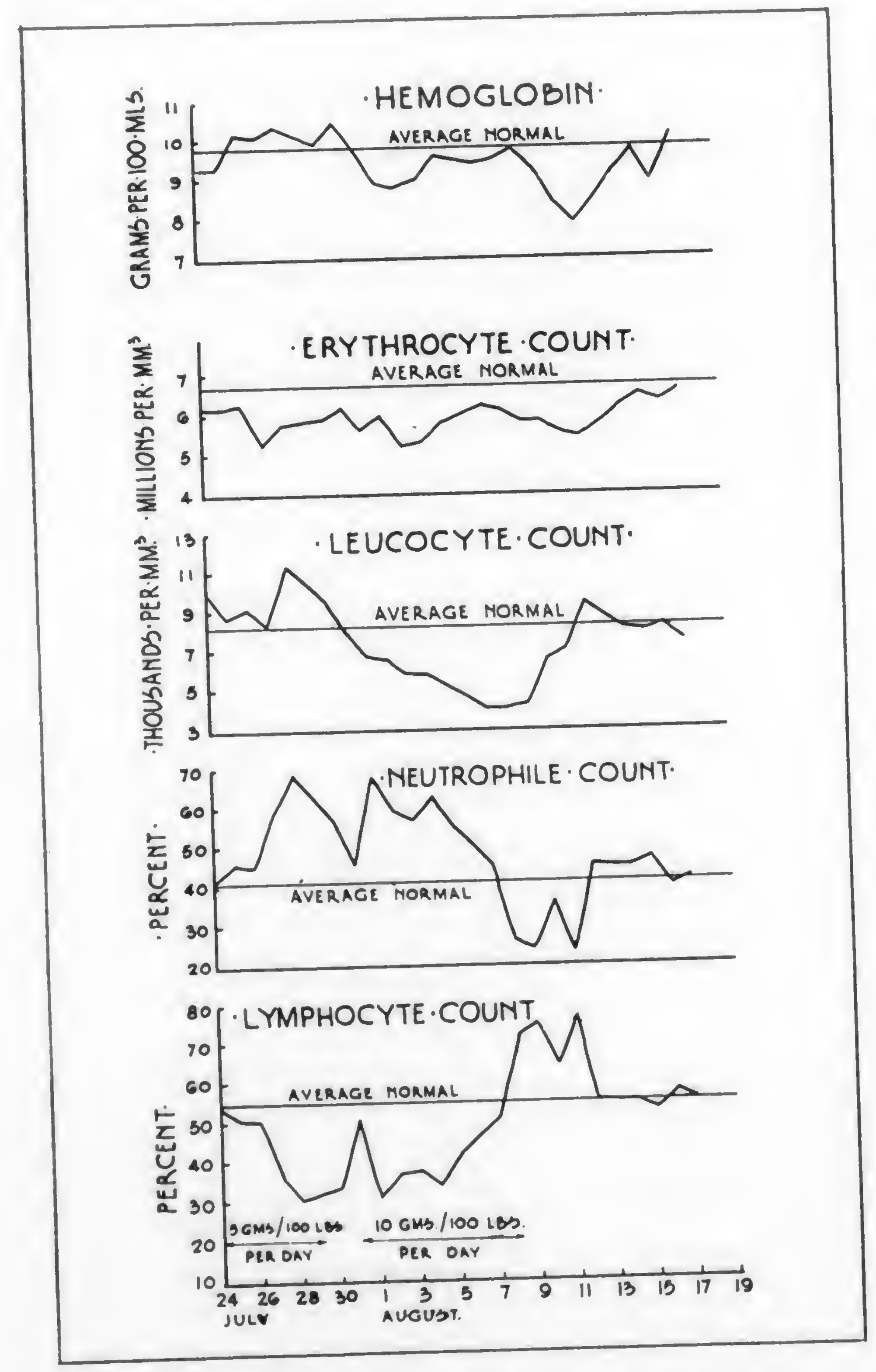


Fig. 2—The effect of sulfanilamide administration on the hemoglobin values and the blood cell counts.

The lowest value noted was 5,220,000 cells per cubic millimeter, representing a reduction of 22 per cent from the normal.

The normal leucocyte count for cow 184 was found to be 8,200. On the low level of dosage, a mild leucocytosis was produced. When the high level of dosage was instituted, there was a marked reduction in the leucocyte count. The response was rapid, a definite leucopenia being produced which reached a minimum of 3,960 per cubic millimeter of blood, representing a reduction of 52 per cent from the normal. After the dosage was discontinued, the leucocyte count rapidly returned to normal.

The normal differential count presented in the following table is the average of nine counts of 200 cells each made on cow 184 during the control period, representing an average based on 1,800 cells. No distinction was made between small and large lymphocytes, and the monocytes also included the so-called transitional cells.

TABLE II.—Showing Differential Leucocyte Count During the Initial Control Period

	PER CENT	TOTAL
Neutrophiles	41	3,362
Lymphocytes	55	4,510
Monocytes	1	82
Eosinophiles	2	164
Basophiles	0	0
Divided lymphocytes	1	82

The only significant change due to the administration of sulfanilamide was found in the proportion of neutrophiles and lymphocytes. There was a definite increase in the number of neutrophiles, which reached a maximum value of 69 per cent. As was to be expected, the lymphocyte count varied inversely with the neutrophile count. A definite lymphopenia was produced, reaching a minimum value of 31 per cent.

During the high level of dosage, atypical and degenerated leucocytes appeared in the blood. These include monocytes with a lobulated nucleus, large atypical monocytes, and lymphocytes in various stages of disintegration. The significance of these cells is not known. The blood studies were continued after sulfanilamide administration was discontinued until approximately normal results were obtained, at which point the investigation was concluded.

The normal values for cellular counts and hemoglobin presented in this paper are in close agreement with those reported in the literature.[4, 6, 12]

The animals were weighed at the conclusion of the study and showed an average loss in weight of 8.6 per cent, as represented in table III.

TABLE III.—Loss of Weight Following Ingestion of Sulfanilamide

COW No.	WT. BEFORE JULY 20 LB.	WT. AFTER AUG. 7 LB.	WT. LOSS LB.	WT. LOSS %
184	1,240	1,150	90	7.3
186	1,265	1,200	65	5.1
204	1,250	1,090	160	12.8
239	1,100	1,000	100	9.1

SUMMARY

Four normal Holstein-Friesian cows were given sulfanilamide on two levels of dosage, 5 and 10 Gm. per 100 pounds of body weight per day. A study was made of the concentration of free and conjugated sulfanilamide in the blood and milk. In addition, a study was made of the hemoglobin values and the blood picture, namely, the erythrocyte, leucocyte, and differential counts.

On the low level of dosage, 69 per cent of the total sulfanilamide found in the milk was present as the conjugated compound, whereas, 37 per cent was present in the blood as the conjugated derivative. On the high level of dosage, 56 per cent of the total sulfanilamide was conjugated in the milk, whereas, little conjugated sulfanilamide could be detected in the blood on the same level of dosage. Free sulfanilamide distributed itself approximately equally in the blood and milk on both levels of dosage.

A slight anemia was produced, with a maximum reduction in the erythrocyte count and in the hemoglobin value of about 20 per cent. On the low level of dosage, a slight leucocytosis was observed, while on the high level of dosage, a marked leucopenia was produced, representing a decrease in leucocytes of 60 per cent. A decided increase in percentage of neutrophiles was noted. In addition, atypical and degenerated leucocytes were found in the blood during the height of sulfanilamide administration.

The milk production was found to vary inversely with the excretion of sulfanilamide in the milk. The milk flow was reduced by as much as 82 per cent.

An average decrease in weight of 8.6 per cent resulted from the ingestion of sulfanilamide.

Individual differences in tolerance towards sulfanilamide were noted in the cows throughout the course of this study.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the Department of Dairy Husbandry for furnishing the experimental animals, and to E. R. Squibb and Sons, New York City, for furnishing the sulfanilamide used throughout the course of this investigation.

References

[1] Allot, A. J.: The Treatment of Bovine Mastitis with Sulfanilamide. J.A.V.M.A., xci (1937), p. 588.

[2] Bauer, H., and Gunderson, M. F.: The Effect of Sulfanilamide on the Streptococci in the Udder of Mastitis Cows and a Method for its Estimation in Milk. J. Bact., xxxv (1938), p. 66.

[3] Bigler, J. A., Clifton, W. M., and Werner, M.: The Leucocyte Response to Sulfanilamide Therapy. J.A.M.A., cx (1938), p. 343.

[4] Brooks, H. J., and Hughes, J. S.: The Hemoglobin Content of the Blood of Dairy Cattle. J. Nutr., v (1932), p. 35.

[5] Gildow, E. M., Fourt, D. L., and Shaw, A. O.: Sulfanilamide in the Treatment of Streptococcic Mastitis. J. Dairy Sci., xxi (1938), p. 789.

[6] Hayden, C. E., and Fish, P. A.: The Normal Blood of Some Domesticated Animals. Report of the New York State Vet. Coll. at Cornell Univ., No. 18 (1927-1928), p. 207.

[7] Jennings, C. H., and Camp, M. B.: Anemia and Agranulocytosis during Sulfanilamide Therapy. Lancet., ii (1937), p. 899.

[8] Kalin, S. L.: Anemia during Treatment with Sulfanilamide. J.A.M.A., cix (1937), p. 1005.

[9] Little, R. B.: The Effect of Sulfanilamide in Bovine Mastitis Streptococci. Cornell Vet., xxix (1939), p. 282.

[10] Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine. J. Biol. Chem., cxxii (1937), p. 263.

[11] Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr.: Acetylation of Para-amino-benzene-sulfonamide in the Animal Organism. Science, lxxxv (1937), p. 202.

[12] McKay, C. H.: The Hemoglobin and Total Phosphorus in the Blood of Cows and Bulls. J. Dairy Sci., xiv (1931), p. 373.

[13] Newcomer, H. S.: A new Optical Instrument for the Determination of Hemoglobin. J. Biol. Chem., lv (1923), p. 569.

[14] Ramsay, A. C.: The Use of Sulfanilamide for Mastitis. Canad. J. Comp. Med., ii (1938), p. 129.

[15] Roemer, R.: A new Treatment for Mastitis. Guernsey Breeders J., liii (1938), p. 331.

[16] Schlotthauer, C. F.: The Use of Sulfanilamide in the Treatment of Mastitis. Intermountain Livestock Sanitary Assoc. (Jan. 1938).

Reprint from Poultry Science: Vol. XXI, No. 6
November, 1942

Feeding-Cane Molasses as a Constituent of Poultry Rations.[*] II. Molasses for Adult Chickens

Walther H. Ott[1] and R. V. Boucher
Department of Agricultural and Biological Chemistry

and

H. C. Knandel
Department of Poultry Husbandry, The Pennsylvania State College, State College, Pennsylvania

(Received for publication April 7, 1942)

In a recent communication (Ott, Boucher, and Knandel, 1942) the performance of Single Comb White Leghorns was reported for the first 24 weeks of age on two experiments in which four all-mash rations varying in feeding-cane molasses content were fed in duplicate from 1 day to 72 weeks of age. The present paper is a report of the performance of these fowls during the laying periods in these experiments.

Previous investigators have reported various results with laying hens fed rations containing cane molasses. Winter (1929), in a six-month trial starting with 240 Leghorn hens and a year trial starting with 60 Leghorn pullets, reported nearly equal feed intake and egg production, lower mortality, and higher water intake with rations containing 5, 10, or 15 percent molasses than with rations containing no molasses. In one 20-week test starting with 100 Leghorns, Maw (1933) noted nearly equal feed intake and change in body weight, lower egg production and mortality, and higher water intake with rations containing 1.25, 5, or 6.25 percent than with no-molasses rations.

In one 8-month and two 10-month experiments starting with 337 Leghorns having access to green feed in yards, Upp (1937) reported nearly equal feed intake, egg production, egg size, and body weight change and increased hatchability, mortality, and water intake when 5, 7.5, or 10 percent molasses was included in the ration. Both Winter and Upp called attention to the laxative properties of the 10 and 15 percent molasses diets. Gericke (1940) mentioned the use of one-half pound of molasses mixed with one gallon of water in preparation of a wet mash to stimulate appetite and egg production.

These variable results tend to indicate that rations for laying hens may safely contain feeding-cane molasses up to the amount that has an undesirable laxative effect.

EXPERIMENTAL

Four all-mash rations containing 0, 2, 4, and 6 percent cane molasses in place of equal amounts of yellow corn were fed in duplicate to Single Comb White Leghorns from 1 day to 72 weeks of age in each of two experiments. During the laying period each group of females received the same all-mash ration they had been receiving from 16 to 24 weeks of age (Ott *et al.*, 1942).

In the first experiment, which ended September 11, 1940, the birds were housed in an open-front type of unheated house. In the second experiment, which ended September 24, 1941, the birds were housed in a building with glass windows in front and equipped with a warm-air ventilation system and sun porches. Artificial morning lights were used during the winter months to provide about 13 hours of light daily. Four males of the college breeding flock were kept in each pen from the beginning of the laying period to the end of the hatching season.

---

[*] Authorized for publication on April 3, 1942, as paper No. 1094 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This investigation was made possible through a fellowship grant by the Commercial Molasses Corporation, New York City.

[1] Now with the Merck Institute for Therapeutic Research, Rahway, New Jersey.

Individual body weights and pen feed intakes were recorded at four-week intervals. Date of first egg was obtained from trapnest records, and body weight at date of first egg was calculated by linear interpolation between the preceding and following regularly obtained body weights. The total egg production in each pen was counted and weighed daily, and from these data the mean egg production and mean egg weight were calculated.

For the hatchability studies, two eggs, each weighing over 1.75 ounces, were incubated from each pullet laying two or more eggs in a five-day collection period each month from January through June. The physical quality of fresh eggs was determined in January, June, and August on two successive eggs from each hen in production at those times, using the method described by Haugh (1937) for determination of the physical quality.

All birds in the eight pens of the first experiment and all birds in one series of four pens and a portion of the birds in the duplicate series in the second experiment were first generations on the experimental diets. The remaining females in the latter series of four pens in the second experiment were progeny of 41 females in the first experiment and received the same diets their parents had received in order to furnish information regarding performance of a second generation on the respective diets.

RESULTS AND DISCUSSION

First Generation

Mean performances of the first generations of laying hens on the four experimental diets are given in Table 1. None of the differences between diet means was statistically significant. Differences between the duplicates were likewise not significant. Differences between the two experiments were significant in egg production (both number and total weight), age and weight at first egg, and hatchability, the results in the second experiment being higher.

The relative rank of the four diets in the first experiment did not differ significantly in any criterion from the relative rank in the second experiment. This was likewise true of the relative rank of the four diets in the duplicate series within each experiment. The interaction between duplicates and experiments was also not significant. For these reasons, the mean performance in each criterion on each diet is given in Table 1. Each tabulated value is the mean of the four pen means on that diet. The number of hens is the sum of the numbers for the four pens on each diet.

Body weight was highest at 48, 52, and 56 weeks of age. The mean of these weights is reported as the maximum body weight in the tables. The June and August values for egg quality for the respective diets were very close together, so they were averaged. Feed intake was highest and mortality was lowest in the groups fed 4 and 6 percent molasses in the ration. Otherwise, performance was nearly equal on the diets containing 0, 4, and 6 percent molasses. Performance of the 2 percent group was inferior in most criteria.

The outward appearance of the birds was the same on all rations. A slight laxative effect was observed on the 6 percent molasses diet. This did not cause any practical difficulty except in the first experiment during damp winter weather when the litter in the 6 percent molasses pens had to be changed about 10 percent earlier than the litter in the no-molasses pens. In reasonably dry or warm weather during the first experiment and under all conditions of weather during the second experiment, no differences were evident in condition of the floor litter.

TABLE 1.—*Mean performances of first generations of laying hens*

Criterion		Percent cane molasses			
		None	2	4	6
Number hens alive at 24 weeks of age		149	156	147	160
Feed intake per hen, 24 to 72 weeks, lbs.		83.28	81.38	86.88	85.32
Body weight of hens alive at 72 weeks of age	initial weight, gms.	1,626	1,609	1,626	1,655
	maximum weight, gms.	1,953	1,878	1,929	1,906
	final weight, gms.	1,841	1,744	1,852	1,770
Egg weight, 48 to 60 weeks, gms.		55.4	54.2	55.0	55.4
Total weight of eggs produced per hen to 72 weeks of age, gms.		9,927	8,867	9,750	9,854
Body weight at date of first egg, gms.		1,822	1,793	1,808	1,792
Age at date of first egg, days		201.2	206.6	204.3	202.2
Egg production per hen to 72 weeks of age		183.6	167.6	180.6	182.5
Physical quality of fresh eggs in Haugh units	Jan.	86.0	86.0	87.2	86.7
	June–Aug.	75.1	76.4	76.6	78.0
Hatchability of fertile eggs, percent		82.9	82.8	82.4	84.6
Mortality, 24 to 72 weeks, percent		28.6	28.1	19.5	24.2
Number hens alive at 72 weeks of age		109	111	117	122

All these facts support the conclusion that the performance of the laying pullets was uniform on the four diets varying in cane molasses content from 0 to 6 percent. In consideration of the management difficulties probably arising from the increased laxative action reported by other investigators for all-mash diets containing more than 6 percent molasses, it appears that the relative costs of ground yellow corn and feeding-cane molasses may be the determining factor in the use of molasses up to 6 percent of an all-mash ration.

Second Generation

Mean performances of 41 females in the first experiment and their 96 progeny fed the corresponding diets in the second experiment were obtained from the individual records of these hens during the laying period and are given in Table 2. These four groups of second generation hens were confined with smaller numbers of first generation hens in one series of four pens in the second experiment. Consequently, performance of the second generation alone could be determined only in those criteria in which individual hen records were available.

In most instances the performances of the two generations on each diet were relatively close together. Differences were appreciable as follows: On the no-molasses diet the second generation was lower in egg quality than the first generation; on the 2 percent molasses diet the second generation was higher in age at first egg and lower in body weight and in hatchability; on the 4 percent diet the second generation was higher in age at first egg and in egg production and lower in hatchability; and on the 6 percent diet the second generation was higher in age at first egg and lower in body weight and in egg production. Mortality on the 4 and 6 percent molasses diets was half as high as on the 0 and 2 percent diets.

Although these observations were made on relatively small numbers of birds and this phase of the experimental work was not conducted in duplicate, the results seem to indicate that the performance of the sec-

TABLE 2.—*Comparative mean performances of first and second generations of laying hens*

	Percent cane molasses							
	None	2	4	6	None	2	4	6
	Dams				Progeny			
	Number of hens alive at 24 weeks of age							
	10	9	13	9	33	22	33	33
	Body weight in grams of hens alive at 72 weeks of age							
Initial	1,710	1,690	1,700	1,830	1,630	1,560	1,610	1,700
Maximum	1,960	2,050	1,970	2,070	2,000	1,890	2,020	1,940
Final	1,860	1,940	1,930	2,050	1,930	1,860	1,960	1,840
	Body weight in grams at date of first egg							
	1,870	1,810	1,880	1,910	1,870	1,840	1,930	1,890
	Age in days at date of first egg							
	197	185	202	185	200	220	220	215
	Number of eggs produced per hen, 24 to 72 weeks of age							
	179	172	166	194	182	166	195	168
	Physical quality of fresh eggs in Haugh units							
Jan.	91	86	88	92	83	91	88	89
June–Aug.	85	81	81	85	78	81	79	83
	Percent hatch of fertile eggs							
	90	85	94	84	88	76	82	85
	Percent mortality, 24 to 72 weeks of age							
					27	27	15	15
	Number of hens alive at 72 weeks of age							
	10	9	13	9	24	16	28	28

ond generation was as satisfactory as that of the first generation on each of the four diets.

SUMMARY

Statistical analyses of data collected on more than 550 Single Comb White Leghorns finishing their first laying period from 24 to 72 weeks of age indicated that performance was not significantly influenced by variation in the feeding-cane molasses content of the ration from 0 to 6 percent. The evidence indicated further that performance of the second generation was as satisfactory as the first generation on diets containing 0, 2, 4, and 6 percent molasses. It appears that the relative costs of ground yellow corn and feeding-cane molasses may be the determining factor in the use of molasses up to 6 percent of an all-mash ration.

REFERENCES

Gericke, A. H., 1940. The feeding of molasses to poultry. Rhodesia Agr. Jour. 37:324-325.

Haugh, R. R., 1937. The Haugh unit for measuring egg quality. U.S. Egg and Poultry Mag. 43:552-555; 572-573.

———,1937. An easy method for finding Haugh units. U. S. Egg and Poultry Mag. 43:760-761.

Maw, A. J. G., 1933. Feeding cane molasses to growing chicks and laying hens. Sci. Agr. 13: 743-745.

Ott, W. H., R. V. Boucher, and H. C. Knandel, 1942. Feeding-cane molasses as a constituent of poultry rations. I. Molasses for growing chickens, Poultry Sci. 21:340-345.

Upp, C. W., 1937. Cane molasses in poultry rations. Louisiana Agr. Exp. Sta. Bul. 289.

Winter, A. R., 1929. Cane molasses for poultry. Poultry Sci. 8:369-373.

Reprinted from SOIL SCIENCE
Vol. 55, No. 1, January, 1943

THE BEARING OF POTASSIUM ON THE QUALITY OF TOBACCO

D. E. HALEY AND J. J. REID
The Pennsylvania State College

Received for publication September 10, 1942

The production of tobacco of high quality is influenced by many factors, among which are the absorption and the utilization of nitrogen and potassium. That these two nutrients profoundly affect the character of growth has long been known, but their role in the production of high-yielding, disease-resistant tobacco of excellent quality is frequently misunderstood.

Many investigators have attributed the lack of resistance to leaf spot diseases in tobacco to excessive nitrogen uptake, but some of the most seriously diseased samples examined in these laboratories have been found to contain considerably less than the normal nitrogen content of the variety. Disease-free tobacco grown on adjacent plots has been found, on the other hand, to possess as a rule the normal nitrogen content of the type.

Certain investigators have attributed disease resistance to adequate potassium uptake, whereas others in recent years have denied that any relation exists between potash fertilization and disease resistance.

The assumption that the three factors, excellence in quality, resistance to leaf spot diseases, and economic yield are incompatible is common among growers of tobacco, although some growers succeed in combining quality and resistance with satisfactory yield year after year.

These conflicts in opinion and the lack of evidence to explain apparent discrepancies in observation led the authors to initiate a research program in this field. This has been conducted for more than five years through plot work, seedbed studies, greenhouse experiments, and chemical and bacteriological laboratory investigations. Extensive field surveys also have been made to gather further data. Data obtained in this program and conclusions drawn therefrom which pertain to the bearing of potassium on quality, yield, and disease resistance are presented briefly in this paper.

In the production of tobacco there are two distinct phases in the culture of the plant in which environmental conditions and cultural practices differ markedly. Seedlings are produced in coldframes, hotbeds, or greenhouses and later are transplanted to the field. Investigation of the culture of the crop, therefore, has been divided into separate studies of tobacco in the seedbed and in the field.

SEEDBED INVESTIGATIONS

In its accepted meaning, "quality" in the seedling has usually covered disease resistance. The quality seedling is free from disease, transplants without great loss, and strikes root readily even under somewhat adverse conditions in the field. "Yield" in the seedbed usually refers to two factors: number of plants produced per square foot of bed, and length of time required to produce a plant suitable for setting in the field.

Thirty years ago the practice of seedbed steaming was introduced in the Pennsylvania tobacco-producing region, and within a few years this method supplanted all others in the preparation of tobacco seedbeds. Although designed to destroy weed seeds, steaming was soon recommended as a necessary sanitary precaution in the fight against seedbed diseases. These diseases became an economic problem in the years immediately following the introduction of seedbed steaming.

Surveys, by the authors, of the tobacco region of Pennsylvania have shown that it has been the practice to incorporate large amounts of fresh manure and large applications of cottonseed meal usually without P and K supplements in the soil prior to steaming. The bed was then steamed and seeded within a short time. The appearance of leaf spot diseases in such beds was the rule, and the plant produced might well be described as soft, succulent, root-deficient, mineral-starved, and nitrogen-gorged. In order to maintain sufficient moisture in the upper layer of freshly steamed heavy soil, it was necessary to water daily and keep the bed tightly covered with cloth to prevent evaporation. An examination of the soil usually showed a uniformly high content of NH_3-nitrogen, little NO_3-nitrogen, a water-logged condition, and excessive reproduction of bacteria within the soil and on the aerial parts of the seedling.

All seedbed soils and all seedling plants examined showed the presence of the bacteria associated with the leaf spot diseases of tobacco regardless of sanitary precautions taken by the grower and regardless of the presence or absence of visible signs of disease.

At this stage in the investigation the authors and their associates made a study of the nature of these bacteria and established that these organisms, *Phytomonas tabaca* and *P. angulata*, were merely transitory physiological adaptations of the ubiquitous soil saprophyte, *Pseudomonas fluorescens*. It was further established that these organisms are not destroyed in seedbed steaming or by other routine sanitary measures followed in the Pennsylvania tobacco area and that the normal tobacco plant is exposed to these adaptations of *Pseudomonas fluorescens* at all times in both seedbed and field[1].

It became evident as a result of the information obtained in this phase of the investigation that the incidence of leaf spot diseases is related not to the presence of the organisms (which are ubiquitous) but rather to the relative resistance of the host to infection.

An investigation of the factors controlling resistance to leaf spot diseases in the seedbed has shown that the tilth and moisture-holding capacity of the soil must be excellent in order to produce a plant with adequate root system and with the minimum use of water. In addition the nutrients supplied must be available in such proportions that the N/K ratio of the seedling is approximately 0.6. Furthermore, beds must be ventilated and photosynthesis must be undisturbed by excessive use of cloth. Under such conditions high-quality, disease-resistant seedlings may be produced economically.

---

[1] Reid, J. J., et al. 1942 Bacterial leafspots of Pennsylvania tobacco. Pa. Agr. Exp. Sta. Bul. 422.

The provision of such environmental conditions is not difficult. Fall-steaming of the previously manured bed permits a return of the soil to approximately normal physical condition through alternate freezing and thawing in the winter months. This eliminates much of the trouble. It is not necessary to water fall-steamed beds excessively or to keep them closed in order to hold moisture. Fall-steaming coupled with suitable fertilization to permit the uptake of sufficient K and other minerals results in the production of high-quality disease-free plants. It has been found that an application of a 4-8-12 or closely allied fertilizer mixture at the rate of 1,000 pounds per acre will suffice for the nutrient requirements of the seedlings.

It is true that there is a definite limit to the number of quality seedlings that can be produced in a given area, and the grower may seed too heavily in an effort to provide more seedlings than the bed space warrants. To this extent quality and excessive plant yield in the bed are incompatible. On the other hand, the difference in seedling size often noted when soft succulent plants are contrasted with normal high-quality plants is more apparent than real. Laboratory studies show that the weight of dry matter produced by the quality plant is as great as that produced by the tall, stringy, succulent one.

FIELD CULTURE OF TOBACCO

In the investigations of field culture of tobacco conducted by the authors, the cooperation of the Chatham Substation of the Virginia Agricultural Experiment Station was obtained. As a result, the research included the field production of Pennsylvania tobacco of the cigar-filler type and also the flue-cured tobacco of Virginia. The aid of leaf experts in the industry was enlisted, and their judgment of quality was obtained on all samples handled. More than 500 samples of flue-cured tobacco and more than 1,000 samples of cigar-leaf tobacco were examined in the chemical and bacteriological laboratories in the course of these investigations. Determinations of the N and K content and of the microflora were made routinely, and in addition, as circumstances warranted, determinations were made of Ca, protein N, nicotine, reducing sugars, steam-volatile acids, and fixed organic acids. These chemical and bacteriological data were related directly to quality in the leaf.

The results showed that flue-cured tobacco of excellent quality contains approximately 2 per cent N, at least 2 per cent K, and 18 to 25 per cent reducing sugars. Any significant divergence from these values was found to be reflected in a lowering of the quality of the leaf.

High-quality cigar-leaf tobacco was found to contain between 3.5 and 4.2 per cent N, slightly more K than N, decidedly less Ca than K, and at maturity, protein N somewhat in excess of nonprotein N. It was also found at maturity to contain somewhat less than 1 per cent of oxalic acid, from 8 to 12 per cent malic acid, and about 2.5 per cent citric acid. These values change during air-curing, but any serious divergence at maturity was associated with lack of quality.

The microflora of flue-cured tobacco was found to be an excellent index of quality, high quality being associated with an active saccharolytic flora, and poor quality with either a proteolytic or inert flora depending on the factors responsible for the lack of quality. In a somewhat similar manner the bacteriological findings in the case of cigar-leaf tobacco were related to the quality.

With this information on the relation of quality to chemical composition and microflora of the leaf as a reference point, attention was turned to the study of fertilizer experiments at Chatham, Virginia, and Roseville, Pennsylvania.

The Chatham studies[2] revealed that under the conditions of the experiment the highest-quality leaf was obtained by the use of three to four units of potash for each unit of nitrogen applied. Typical analytical data on tobacco leaves from a high-quality plot follow:

YEAR	NITROGEN	POTASSIUM	REDUCING SUGARS
	per cent	*per cent*	*per cent*
1938	2.07	2.17	22.18
1939	1.93	2.43	22.58

Throughout a 4-year study of these plots it was found that smaller applications of potash resulted in a lowering of quality, of market value per acre, and of K and reducing-sugar content and an increase in nitrogen content, although nitrogen additions were the same in all plots in the series. Larger applications of potash resulted in a slightly lower N content, a slightly higher K content, with but little change in reducing sugar content, and slightly less monetary return per acre.

Tobacco grown with three or more units of K_2O per unit of N was uniformly free from leaf spots of all types during the course of the study, although those plots receiving less K_2O exhibited leaf spots physiological and bacterial in nature.

The studies of the Chatham tobacco included separate analyses of lower, middle, and upper leaves from each of the plots included in the program. This revealed a significant fact in the chemical composition of the plant. All treatments with less than three units of K_2O per unit of N resulted in the production of a plant with less potassium in the lower leaves than in the upper part of the plant. This condition was always found to be associated with inferior quality not only in the K-deficient lower leaves but in the upper leaves as well. Treatments giving the highest quality resulted in a uniform distribution of potassium throughout the leaves. Excessive K_2O fertilization resulted in a greater accumulation of K in the lower leaves than in the middle and upper leaves. It would appear, therefore, that potassium deficiency actually exists in any tobacco plant that contains at maturity less potassium in the lower leaves than in the middle and upper leaves and that such deficiency is associated with a definite lack of quality in all leaves of the plant. This work indicates that such a deficiency is also associated with susceptibility to leaf spotting, which is frequently economic in severity.

---

[2] Gribbins, M. F., Reid, J. J., and Haley, D. E. 1941 The distribution of potassium in bright leaf cigarette tobacco and its influence on the quality of the leaf. *Jour. Agr. Res.* 63: 31–39.

The problems confronting the grower of cigar-leaf tobacco on the heavy soils of Lancaster County, Pennsylvania, differ from those of the Virginia grower of flue-cured tobacco. The yield of cigar-filler leaf must be far larger in terms of pounds produced per acre than in the case of flue-cured tobacco, if a profit is to be realized. In addition the cigar leaf must contain approximately twice as much nitrogen and potassium as does the flue-cured leaf. In order to obtain large yields of the cigar-leaf tobacco, either heavy applications of animal manure or extensive green manuring supplements the use of commerical fertilizers on most tobacco land. Outbreaks of leaf spot diseases have constituted an ever-present threat to the Pennsylvania crop during the last 25 years, and yield and quality on the average farm have varied greatly with climatic conditions obtaining from year to year.

Dry-weather crops of cigar-leaf tobacco are poor in quality, and the leaf is heavy, gummy, and difficult to ferment. Potassium content is usually low and Ca content high. Yields are not satisfactory in these dry seasons, but disease in economic proportions does not appear. The crop of 1929 is an example of such tobacco.

Wet-season crops, although not of the best, are superior in quality to dry-season crops, but the leaf is thin and the yield is not always satisfactory. Contrary to popular opinion, disease is of little significance in wet seasons if there has been sufficient moisture to maintain growth at all times. The crop of 1942 furnishes an excellent example of tobacco produced in a truly wet season.

Seasons in which the amount of rainfall is ideal and distribution equitable are infrequent, but the crop of 1936 furnishes an example of tobacco produced under such conditions. Yields are large, quality is good under proper fertilization, the leaf ferments well, and the chemical composition shows K in excess of N in the best of the crop. Leaf spot disorders do not occur in such years.

The season of erratic rainfall distribution with periods of drought and periods of excessive rainfall interspersed constitutes the fourth and perhaps the most prevalent type. These conditions result in inferior quality in the bulk of the crop, often poor burn and poor fermentability, and widespread loss from leaf spot diseases. Such tobacco is relatively high in nitrogen and low in potassium and contains abnormally large amounts of calcium and oxalic acid.

In spite of these wide fluctuations in the bulk of the Pensylvania crop due to climatic conditions from season to season, some growers year after year produce good tobacco with good yields and no economic loss from disease. Likewise, on certain plots of the Pennsylvania Agricultural Experiment Station similar tobacco has been produced, although adjoining plots yielded small amounts of inferior leaf, riddled in many cases with leaf spot diseases.

On the basis of this knowledge, extensive surveys were made by the authors and additional plot work was undertaken. Having previously determined that the bacteria associated with leaf spots constituted a normal part of the plant flora and that leaf spot incidence, therefore, was in no way related to the mere presence of the epiphyte, the authors were in a position to study the epidemiology of the leaf spot diseases to determine why certain plants succumbed while others lived in health with the organism as a harmless saprophyte on the leaf.

From the field surveys and chemical analyses of tobacco procured from growers in addition to crops from the experimental plots it was evident that disease resistance invariably accompanied excellence in quality and that such tobacco contained at least as much K as N. On the other hand, this information could not be directly related to K fertilization, as many plots and fields heavily fertilized with K failed to produce either high quality or disease resistance under erratic rainfall. Chemical analyses showed, however, that these plants had failed to assimilate adequate amounts of K, although tests showed its availability in the soil at the end of the growing season. For example, plots in the 4-year rotation in 1937 which received at least 1,000 pounds per acre of a 3-8-12 fertilizer yielded tobacco of high quality and with insignificant leaf spotting, whereas plots in the 3-year rotation with equivalent fertilization suffered more than 20 per cent loss from leaf spot diseases and the tobacco remaining was poor in quality and yield. Analyses revealed that the tobacco in the 4-year rotation had taken up almost as much K as N whereas that in the 3-year rotation had absorbed only about half as much K as N. Examination of root systems showed that the plants on the 4-year rotation had developed almost normally, whereas the roots of the plants of the 3-year rotation plots were much less extensive. The initial handicap of the soil under the two systems of rotation was determined by a study of the check plots and is very pertinent to the entire problem. These data are briefly summarized as follows:

ROTATION	NATURE OF LAND	LEAF SPOT DAMAGE	YIELD PER ACRE	SALABLE TOBACCO PER ACRE
		per cent	*lbs.*	*lbs.*
4-year	Untreated corn land	11.9	1008	888
3-year	Untreated clover land	31.2	1092	751
3-year	Untreated alfalfa land	35.7	1176	756

It is obvious from these data that a given treatment had a much greater handicap to overcome on the legume sodland than on the stalkland.

Further plot studies, based on this information, showed that in those years of erratic precipitation with drought followed by excessive rainfall, the tobacco plant deteriorated in quality and in disease resistance in accordance with the extent to which it suffered from interruptions in growth followed by tremendous N uptake at the break in the drought. Physiological youth of some plants would save them from disaster whereas those undergoing such interruptions while physiologically old invariably suffered severely. Soil drainage, tilth, rotation, manurial treatments, and cultivation as well as commercial fertilization determine the extent to which a plant may continue to grow and develop normally under adverse conditions.

For proper tilth and moisture-holding capacity in the heavy soils of Lancaster County, Pennsylvania, a liberal supply of humus is necessary. This need cannot be met by the incorporation of old plant residues or of fresh animal manures if there is insufficient moisture to accomplish the conversion of this raw material to humus. The plant living over a bed of fermenting organic material is usually suffering from microbial competition not only for moisture, if rainfall is scant, but also, in the early stages of growth, for nitrogen if the organic material is deficient in readily available nitrogen. Far from supplying the moisture-holding capacity of humus, these raw materials actually act in the opposite direction until humification has been accomplished in large measure.

The solution to the question of the lack of proper nutrient absorption by tobacco on old legume sodland in years of erratic precipitation is, therefore, a simple one. Such soil does not provide a moisture reserve for uninterrupted growth. Moreover, it contributes the most N to the plant not when needed in such seasons but rather when the plant is unable to take care of excessive N absorption. On a soil manured with unfermented animal manure, fermentation is somewhat more readily accomplished and N deficiency in the early part of the season is less likely to occur.

Further investigations have shown that any condition which restricts root development or deprives a plant of sufficient moisture in time of drought leads to growth interruption, poor K uptake, N uptake at the wrong period, inferior quality, and susceptibility to disease.

In order to ensure utilization of applied potassium, with consequent improvement in quality and increase in disease resistance, the tilth and moisture relationships of the soil must be considered and adequate provision made through cultural practices to maintain the soil in good tilth and provide an adequate moisture-holding capacity so that growth may continue under adverse conditions. No tobacco plant is any better than its root system, and only in this manner may a normal root system be developed in unfavorable seasons.

Practices which provide such assurance of continued growth, adequate potassium uptake, and the quality and resistance which this guarantees, may be briefly summarized as follows:

> The site must be selected with respect to good drainage. Good tilth must be provided. Humus must be supplied, preferably through the application of well-rotted animal manure, but if this is unavailable green manure may be substituted. It must be plowed under in the succulent state if a crop is to be planted at once. Rotations should be arranged so that the tobacco crop is never transplanted to a field in which old legume residues have just been plowed under. Cultivation should be adequate and should not damage or restrict root growth. Fertilizer applications should be sufficient to meet the demands of the crop and should include at least three times as much potash as nitrogen. The method of application should promote rather than restrict root development.

The experience of practical growers who have followed this system, and the results of experimental plot work, indicate that in this manner good yields of excellent quality tobacco, free from disease, may reasonably be expected in the face of climatic conditions that prove disastrous to the crop in general.

SOIL COMPOSITI
RELATION TO A

Minera
Show V

By ROBERT V. BOUCHER and D. E. HALEY
Department of Agricultural and Biological Chemistry
Pennsylvania State College
State College, Pa.

THAT hay was hay and corn was corn as far as their feeding value in livestock production was concerned was an idea prevalent not many years ago. Apparently, the only thing considered of importance in crop production was the yield. That viewpoint is rapidly changing, thanks to the increased knowledge of plant and animal nutrition and a growing understanding of the influence of soil fertility on the nutritive value of grain, hay and other crops.

It is a recognized fact that animals must depend, either directly or indirectly, upon plants for all of their food. Plants must in turn depend upon the soil, water and air for their well-being. Furthermore, it is known that the elemental composition of the soil itself is in large measure influenced by the parent rock. It is now recognized that an excess or deficiency of a given soil element will not only interfere with normal plant development but animal development as well, provided the vegetation of the given locality constitutes the main source of nutrients for the animals. Many animal disorders are now attributed to the character of the vegetation grown in an area, in which the animals are produced. It is well known that farm animals restricted to natural forages reflect in their rate of growth, appearance, physical condition and performance, not only the nutritive value of the forages but the fertility of the soil as well. For example, the excellence of animals produced in such areas as the Blue Grass Region of Kentucky reflect a high degree of soil fertility, while in other localities the farm animals may appear less thrifty and produc'
in those p

Due t
discuss
nutr
ma
r

fertilizer treatment will not necessarily do so under conditions verging on drought or on too heavy rainfall. Certain elements are leached from these soils under the latter conditions. On the other hand, the intake of fertilizer elements especially potassium, is seriously interfered with where the moisture conditions are low. Moreover, a drought may interfere seriously with the physiological balance of nutrients, a condition which

In comparison wit
cient areas in this c
low-calcium forage is
ception is the acid s
cows on pasture wit
to such an extent that
these conditions mil
rapidly decline.

There are many s
which the calcium su
are such that legum
content of plants is l
due to a deficiency
plant being unable
calcium content of p
by application of fe
not the soil reactio
calcium use by the p

IRON,

These elements hav
in animal nutrition
absence of either o
results. In cattle, nutr
for many years. In F
Holland as "lecksuc
South Africa as "nak
sickness" and in Ma
instances a lack of i
area has been found
shortages of copper
factors in some areas
by adding the miner
better by applying
disease is characterize
of appetite followed
varying severity.

An interesting fac
is that to date shee
that have shown c
healthy for generatio
ill in four months.
cient soils occur in s
New Zealand. The s
appears to be an ex
soil and plants in the
in all respects except

Iodine is an elem
quantities that is o
effect on man and a
portance of idoine in
types of human goit
plishments of medic
goiter in some regi
Wisconsin, Minneso
and North Dakota. H
a very low iodine c
soils are generally
the power of combi
that may have be

SOIL COMPOSITION HAS DIRECT RELATION TO ANIMAL HEALTH

Mineral Nutrients of Farm Crops Show Variations Throughout Country

By ROBERT V. BOUCHER and D. E. HALEY

Department of Agricultural and Biological Chemistry
Pennsylvania State College
State College, Pa.

THAT hay was hay and corn was corn as far as their feeding value in livestock production was concerned was an idea prevalent not many years ago. Apparently, the only thing considered of importance in crop production was the yield. That viewpoint is rapidly changing, thanks to the increased knowledge of plant and animal nutrition and a growing understanding of the influence of soil fertility on the nutritive value of grain, hay and other crops.

It is a recognized fact that animals must depend, either directly or indirectly, upon plants for all of their food. Plants must in turn depend upon the soil, water and air for their well-being. Furthermore, it is known that the elemental composition of the soil itself is in large measure influenced by the parent rock. It is now recognized that an excess or deficiency of a given soil element will not only interfere with normal plant development but animal development as well, provided the vegetation of the given locality constitutes the main source of nutrients for the animals. Many animal disorders are now attributed to the character of the vegetation grown in an area, in which the animals are produced. It is well known that farm animals restricted to natural forages reflect in their rate of growth, appearance, physical condition and performance, not only the nutritive value of the forages but the fertility of the soil as well. For example, the excellence of animals produced in such areas as the Blue Grass Region of Kentucky reflect a high degree of soil fertility, while in other localities the farm animals may appear less thrifty and productive, indicating a low degree of soil fertility in those particular areas.

Due to the limits of the space allowed, the following discussion will necessarily be limited to the six mineral nutrients of greatest practical importance to the feed man and his customer, the farmer. The story of the relationship of soil fertility to vitamin and protein content of crops had best wait for the results of future experiments. Likewise, a discussion of the influence of climate and genetics must be omitted.

PHOSPHORUS

There are large areas in many countries where serious losses in livestock are the result of the low phosphorus content of forage crops. This abnormally low phosphorus content is due to conditions which include lack of phosphorus in the soil and just as frequently conditions which prevent the phosphorus from being taken up by the plant although it may be present in relative abundance. This latter condition exists in South Africa where great losses to the livestock industry were found to be due to the fact that only about 1 per cent of the total phosphorus in the soil was in a condition that could be used by the plants.

There is an interesting situation existing in the Central Basin of middle Tennessee where there are extensive areas of unusually fertile soils. The soil on the hills surrounding this area contain about one-sixth as much phosphorus as the soil in the Basin. It was found that lespedeza hay grown in this area contained 0.27 per cent phosphorus while the same hay cut at the same stage in fields on the surrounding hills contained only 0.1 per cent phosphorus. When steers were fed clover hay from these two localities, those fed hay grown in the Basin gained 50 per cent faster than those fed from the other district.

In grazing areas located in some parts of Florida, the low phosphorus content of both the soil and the forage does not account for all of the deficiency diseases in livestock. The yield of forage may be low and it may likewise be deficient in important nutrients other than phosphorus, resulting in serious losses in the livestock industry.

The phosphorus deficiency disease in cattle is known as "stiffs" or "sweeny." A common symptom is a craving for materials such as bones or oyster shells. The cattle become stiff and lame, lose weight and the cows give less milk. Some die while others are weakened to the extent that they are more susceptible to other diseases.

In this country phosphorus deficient areas are known to exist in Alabama, Florida, Michigan, Minnesota, Montana, Tennessee, Texas and Wisconsin. The condition can be corrected by feeding bone meal or by proper soil management directed at releasing the phosphorus already present or by the addition of phosphorus in the form of fertilizer.

Phosphorus deficiency is not limited to range or pasture-fed animals for it does occur in barn or lot feeding when the feed has been grown on phosphorus poor soil or when poor feeding practices are followed. It is not always realized that the best roughage is not a rich source of phosphorus nor that all concentrates are not good sources of this element. During rapid growth or high milk production special care must be taken to select feeds that will make the ration adequate. This was emphasized by a recent investigation of phosphorus deficiency in Pennsylvania. No areas of phosphorus deficient soil were found but some of the roughages being fed such as corn stover, timothy hay and wheat straw were generally low in this element. It was concluded that phosphorus deficiency should never occur if good feeding practices are followed; such practices include the use of better roughage in place of stover and straw and the selection of concentrates rich in phosphorus.

(Reprinted from the 1943 FEED BAG RED BOOK)

CALCIUM

In comparison with the numerous phosphorus deficient areas in this country, there are few areas where low-calcium forage is known to cause trouble. An exception is the acid sandy soil of Florida where dairy cows on pasture withdraw calcium from their bones to such an extent that they can be readily broken. Under these conditions milk production and general health rapidly decline.

There are many soils, throughout the country, in which the calcium supply, the acidity or other conditions are such that legumes will not grow. The calcium content of plants is lowest on acid soils, not necessarily due to a deficiency of calcium in the soil but to the plant being unable to use that which is present. The calcium content of plants may or may not be increased by application of fertilizer, depending on whether or not the soil reaction and rainfall are favorable for calcium use by the plants.

IRON, COBALT AND COPPER

These elements have this in common, they are required in animal nutrition in very small amounts and in the absence of either one of them, nutritional anemia results. In cattle, nutritional anemia has been recognized for many years. In Florida it is known as "salt-sick," in Holland as "lecksucht," in Scotland as "pining," in South Africa as "nakuruitis," in New Zealand as "bush-sickness" and in Massachusetts as "neck ail." In most instances a lack of iron in the soils and crops of the area has been found to be the cause of the disease but shortages of copper and cobalt may be contributing factors in some areas. The condition may be corrected by adding the minerals directly to the feed or perhaps better by applying them as fertilizer to the soil. The disease is characterized by an unthrifty condition, failure of appetite followed by loss of weight and anemia of varying severity.

An interesting fact that applies specifically to cobalt is that to date sheep and cattle are the only animals that have shown cobalt deficiencies. Horses remain healthy for generations on pasture where sheep become ill in four months. The most pronounced cobalt deficient soils occur in some coastal areas of Australia and New Zealand. The so-called "coast disease" in Australia appears to be an example of single deficiency for the soil and plants in the affected areas seem to be adequate in all respects except cobalt.

IODINE

Iodine is an element occurring in the soil in small quantities that is of great importance because of its effect on man and animals. The discovery of the importance of idoine in the diet for the control of certain types of human goiter is one of the outstanding accomplishments of medicine. There is a high incidence of goiter in some regions, namely, parts of Switzerland, Wisconsin, Minnesota, Washington, Michigan, Idaho and North Dakota. High incidence of goiter accompanies a very low iodine content of the soil. Iodine-deficient soils are generally quite acid and they do not possess the power of combining with and retaining the iodine that may have been there originally. They may contain only a few parts of iodine per billion parts of soil, which is not enough to grow plants with sufficient iodine content to prevent goiter in the animals that eat the forage and grain produced on such soil.

The birth of hairless pigs has resulted from feeding brood sows diets low in iodine. Goiters have been produced in calves and chickens by feeding rations low in iodine. In some cases the application of small quantities of iodine to the soil has resulted in a slight increase in crop yield, but the main value of such additions would seem to be the increase in the iodine content of plants that may supply the element to livestock and directly or indirectly through meat, poultry and dairy products to man. Iodine is now widely added to human diets and livestock rations by incorporating it into the salt.

RELATION OF FERTILIZERS TO SOILS

The effectiveness of fertilizer application and the response obtained is governed to a large extent by a number of factors which include:

1. The Nature of the Soil.—In the weathering of the parent rock, under certain tropical conditions, bases such as potassium, in combination with silicic acid, may be leached to lower levels, leaving a residue of iron and aluminum oxides. Such soils do not hold bases such as calcium to any appreciable extent but may form combinations with phosphoric acid which are practically unavailable to the plants grown thereon. In this connection, it can be stated that this tendency may be observed in many other types of soils as well. An acid soil containing soluble iron may render the phosphoric acid unavailable for plant growth. Regulation of the acidity of the soil by means of additions of lime will tend to overcome this condition. However, over liming tends to render the phosphoric acid unavailable as well. Therefore, a reaction slightly on the acid side of the neutral point is most desirable in this particular instance.

A far different condition of weathering may be noted in certain arid regions. Here is found evidences of weathering with but little if any leaching. This condition has served to build up inorganic base-exchange complexes which tend to hold alkaline materials against the forces of leaching, once these soils are placed under irrigation. However, these soils are quite fertile and do not, as a rule, require extensive fertilizer additions. If such additions are made, the nutrients should be readily available for plant growth.

In certain humid regions, the influence of organic matter is noted in the weathering of the soils. Here may be found a residue of sand in the upper layers and the normally occurring iron and aluminum carried to lower levels. As a rule, however, such soils contain relatively large quantities of both clay and silt. Under proper conditions of soil management, such soils should respond in large measure to a given fertilizer treatment.

2. Soil Acidity.—Agricultural crop plants, as a rule, require a soil slightly acid in reaction for normal growth and development. If allowed to become too acid, there may be a deficiency of both iron and phosphoric acid as well as calcium. Usually, these are calcium deficient soils and it is known that plant growth on such soils, when consumed by animals leads to disorders. The reaction of such soils should be controlled by liming if a response is to be expected from a given fertilizer treatment.

3. Soil Moisture.—An agricultural soil which under normal conditions of rainfall should respond to a given fertilizer treatment will not necessarily do so under conditions verging on drought or on too heavy rainfall. Certain elements are leached from these soils under the latter conditions. On the other hand, the intake of fertilizer elements especially potassium, is seriously interfered with where the moisture conditions are low. Moreover, a drought may interfere seriously with the physiological balance of nutrients, a condition which may prove detrimental to the plant as well as to the animal that depends upon the plant for its food supply.

4. Organic Matter.—In large measure the absorption of nutrients necessary for growth of plants is governed by the extent of root development. Mineral soils may be more or less impervious to the entrance of both air and water which often results in stunted root development, with limited powers of absorption. The addition of organic matter, followed by its decomposition, may lead to a marked binding effect, forming relatively stable combinations where the finer particles are united to larger soil particles. Such a condition tends to insure the proper response of the soil to a given fertilizer treatment.

The longer a soil is cultivated the greater the chance for deficiencies to arise. When the soils of the corn belt areas of the United States were first placed under cultivation, good crop growth of high nutritional value was possible to obtain without the use of fertilizers. As time went on, however, attention was drawn to many deficiencies arising in this and other cultivated parts of the world. In many cases there are sufficient quantities of a given element to insure crop growth but not for the production of plants of a high nutritional value.

As stated before, our attention in the past has been centered on crop yield rather than quality production. Some say that yield and quality may not be compatible but this is not true.

In conclusion it may be said that all of these relationships between soil fertility and nutritive value of plants are of importance not only to livestock but to man as well. There are regions where goiter, skin diseases, weak and deformed bones, mottled teeth and nervous disorders are common. It is recognized that many of these diseases are the result of restricted diets or of eating plants and animals produced on soils either deficient in certain elements or containing others injurious to health. Thus it is plain that plants, animals and man depend first, last and always on the soil for life and health.

[Reprinted from FOOD RESEARCH, 1943, Vol. 8, No. 1, Pages 78-87]

RELATION OF HYDROGEN-ION CONCENTRATION TO COLOR DEVELOPED IN CURED PORK[1, 2]

P. C. DUISBERG AND R. C. MILLER
Department of Agricultural and Biological Chemistry, Pennsylvania State College, State College, Pennsylvania

(Received for publication, July 22, 1942)

The color of cured meat is generally recognized as an important factor in the determination of its market value even though it has not been demonstrated that the color actually affects the quality of the product.

Since the work of Haldane (1901), Hoaglund (1914), Günther (1921), and Urbain and Jensen (1940), it has been known that the red pigments of cured meat, nitric oxide myohemoglobin and nitric oxide hemoglobin, are formed by the myohemoglobin or hemoglobin respectively in the meat muscle, and the nitrite of the curing pickle. That the quality and uniformity of the color developed in the meat by this reaction is affected by other factors has been definitely established. The following list contains the known or suspected factors influencing the color development in cured meat:

1. Nitrite absorbed by the meat. (Enough nitrite must be present to form sufficient nitric oxide myohemoglobin to give a desirable color.)
2. Amount of myohemoglobin or hemoglobin in the tissue. (Enough myohemoglobin or hemoglobin must be present to produce a desirable color shade after the reaction with nitrite.)
3. Microorganisms. (Effect of nitrate reducing bacteria is important in nitrate cures in producing sufficient nitrite for the pigment reaction. Secondary effects of microorganisms in nitrite cures are rather obscure, but probably may enhance the reaction by helping to maintain reducing conditions or hinder reaction by oxidizing meat pigments.)
4. Sugars in the presence of microorganisms. (Sucrose is converted to reducing sugars by microorganisms which help maintain the reducing conditions in the meat necessary for normal nitric oxide myohemoglobin formation.)
5. Atmospheric oxygen. (May affect the color by oxidizing nitric oxide myohemoglobin to the brown pigment metmyohemoglobin.)
6. Ionic concentration of the pickle solution. (May speed up oxidation by lowering the pH.)
7. Oxidation-reduction potential of the meat. (Normally, the reducing conditions necessary for proper formation of nitric oxide myohemoglobin are present in the meat.)
8. Structure of the meat muscle. (Structure and arrangement of the muscle fibers may give slightly different optical properties and produce color differences.)
9. Temperature of curing. (Influence obscure; however, indirectly high temperature is an undesirable factor, since it favors accelerated bacterial growth.)
10. Desiccation. (Affects optical properties of the meat [reflection, etc.] and so affects color.)
11. pH. (Has effect on speed of nitrite-myohemoglobin reaction. Controversial evidence regarding effect on color.)

---

[1] From a thesis submitted by P. C. Duisberg in partial fulfillment of the requirements for the Degree of Master of Science in Agricultural Biochemistry.

[2] Authorized for publication on June 13, 1942, as paper No. 1113 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Among these factors, the data concerning the relationship between pH and color development are particularly scant and inconclusive. Moulton (1936) and Callow (1930) obtained indirect evidence of a relationship between the pH and the color of cured meat. Winkler, Hopkins, and Thistle (1940), using bacon sides, found a moderate degree of association between color quality and pH. Urbain and Jensen (1940), on the other hand, using buffered solutions of nitric oxide hemoglobin prepared from hogs' blood, found no significant spectrophotometric differences over a pH range of 5.1 to 8.

In view of the economic importance of obtaining desirable color formation in cured meats the present study was planned to obtain further data dealing with the effect of pH on color development in cured meat.

EXPERIMENTAL PROCEDURE

In order to study the relationship between the pH and the color developed in cured meats, it was necessary to develop a laboratory method of curing which would approximate actual curing conditions as closely as possible and in which the known variable factors in meat curing could be controlled. In order to minimize any variation owing to differences in the meat and the method of curing, a standard method of sampling and of curing was adopted.

Pork loin was cut into one-inch strips perpendicular to the rib bones. Fat deposits were trimmed away and one-inch cubes, weighing approximately 20 grams, were cut. Only samples of pork between pH 5.6 and 5.7 were used for curing. The pork samples were placed under a General Electric Germicidal Lamp for 10 minutes and then turned over with sterile forceps for an additional 10 minutes. At the same time, 25 milliliters of pickle solution were pipetted into a glass curing jar (height, three inches; diameter, one and one-half inches; having a ground glass stopper) and radiated under the lamp for 20 minutes. The pork was immersed in the pickle solution (with the forceps), the ground glass cover attached, and the samples stored in the refrigerator at 4°C.(39.2°F.).

After a curing period of 24 hours the moisture, salt, and nitrite contents were found to be within the limits found in cured pork and the color and flavor were characteristic of cured pork.

The pickle solution contained 24 per cent NaCl, nine per cent sucrose, .49 per cent $NaNO_3$, and .057 per cent $NaNO_2$, and had a salinometer value of 82° at 25°C.(77°F.). The pickle was made from C. P. salts dissolved in distilled water.

The growth of microorganisms was inhibited by placing the samples under the Germicidal lamp even though the work of Haines (1937) showed that the effect of microorganisms could not be great in curing periods of 24 hours at low temperatures. A subsequent study showed that in the described procedure there was no significant variation in the pH of the pork or the pickle after as much as seven days' storage.

Owing to the use of sucrose and the control of microorganisms, there was no positive test for reducing sugars with Fehling's solution until about the seventh day of curing. Therefore, the combined effect of microorganisms and sugars was controlled by the method of curing.

The pH measurements on the pork were made with a plunging glass electrode, and on the pickle with a glass electrode.

The moisture and nitrite contents were determined by the method of the A. O. A. C. (1935).

The NaCl was determined by the wet digestion method of Davies (1932).

Color was determined with a Bausch and Lomb reflectance spectrophotometer.

It was necessary to determine the normal changes in pH of the curing meat during the time of cure before studying any relationship of color. This study was extended for a period many times longer than the 24-hour cure. The results are illustrated (Fig. 1).

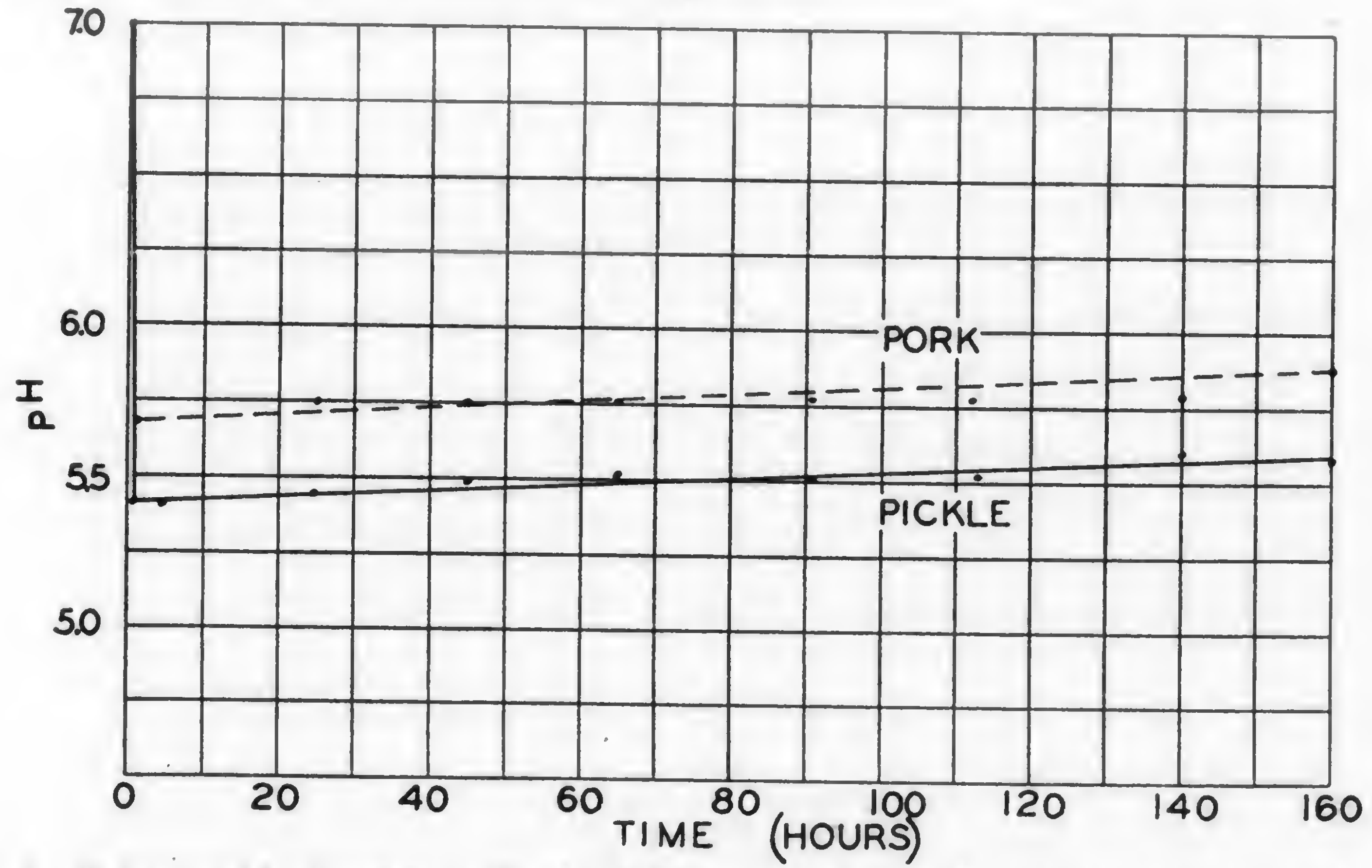


FIG. 1. Relationship between pH of pickle solution and of pork and the time of cure.

It was found that the normal range of the pH of the curing pork remained unchanged at between pH 5.6 and 5.7 for many days. The pH of the pickle solution, after dropping sharply in the first few minutes from near neutrality to about 5.4, then increased slowly to about pH 5.65, a value slightly lower than the pH of the curing pork which increased slowly throughout the period of observation.

It was possible to change the pH of the curing pork and the pickle and still approximate these normal pH relationships between the pork and pickle by curing in buffered solutions. A series of acetate, phosphate, and ammonium buffer solutions, covering a pH range from pH 2 to 10.5, were prepared from the solutions given (Table 1) and added to the pickle

TABLE 1

Concentration of Buffer Compounds

Compound	Normality
CH_3COOH	4 N
CH_3COONa	2 N
K_2HPO_4	2 N
KH_2PO_4	2 N
NH_4OH	4 N
$(NH_4)_2SO_4$	4 N

solution. The resulting changes in ionic strength of the pickle solution from its normal value of 4.14, varied from 3.61 to 4.59. However, subsequent spectrophotometric results on pork cured at pH 5.60 with a buffered pickle solution at an ionic strength of 3.52 and the unbuffered pickle of ionic strength 4.14, indicated that this difference in ionic strength had no effect upon the relationship between the pH and color. Furthermore, this experiment indicated that the phosphate buffer salts added to the pickle had no effect on the pH color relationship. Similar experiments proved that neither the acetate buffer solutions, the ammonia buffer solution, nor various acid bases and salts including HCl, HNO_3, tartaric, lactic, succinic, propionic, phosphoric, and acetic acids, sodium bicarbonate, sodium bisulphate, and sodium hydroxide in various concentrations had any specific effect on color independent of pH.

TABLE 2

Effect on pH of Cured Pork of Addition of Two Milliliters of Buffer Solution to Pickle[1]

Buffer	Molar ratio	Original pH of pickle	After 1-hour cure		After 24-hour cure		After 9-day cure		Original pH of pork
			Pickle pH	Pork pH	Pickle pH	Pork pH	Pickle pH	Pork pH	
$\frac{HAc}{NaAc}$	32-1	2.90	3.60	4.85	3.95	4.60	4.50	4.60	5.40
$\frac{HAc}{NaAc}$	1-1	4.50	4.60	5.10	4.70	5.15	4.90	4.90	5.40
$\frac{HAc}{NaAc}$	1-32	6.00	5.50	5.45	5.50	5.60	5.50	5.65	5.40
Control	....	6.95	5.30	5.50	5.35	5.50	5.50	5.55	5.40
$\frac{KH_2PO_4}{K_2HPO_4}$	32-1	4.10	4.60	4.60	5.00	5.60	5.10	5.45	5.75
$\frac{KH_2PO_4}{K_2HPO_4}$	1-1	5.60	5.80	5.90	5.90	5.90	5.80	5.90	5.75
$\frac{KH_2PO_4}{K_2HPO_4}$	1-32	8.15	7.00	5.95	6.85	6.05	6.70	6.70	5.75
Control	....	6.95	......	......	5.60	5.80	......	......	5.75
$\frac{NH_4OH}{(NH_4)_2SO_4}$	32-1	9.45	8.85	6.45	8.05	6.05	7.00	7.05	6.20
$\frac{NH_4OH}{(NH_4)_2SO_4}$	1-1	8.50	7.95	6.35	7.20	6.50	6.55	6.50	6.20
$\frac{NH_4OH}{(NH_4)_2SO_4}$	1-32	7.30	5.90	6.05	5.80	5.95	5.80	5.90	6.20
Control	....	6.95	5.50	5.75	5.57	5.80	5.75	5.80	6.20

[1] Size of samples—20 grams, one-inch cubes; pickle solution used—25 ml. pickle solution plus two ml. buffer solution; pH of unbuffered pickle solution, 6.95; temperature, 5°C.(41°F.); control of microorganisms—30 minutes under General Electric Germicidal Lamp; salinometer reading of pickle—82.5° at 25°C.(77°F.).

By this method of buffering the pork, it was possible to adjust the pH of the curing pork to an almost constant value in the pH range of 4.4 to 9

within one hour, even when using only two ml. of buffer solution and 25 ml. of pickle solution (Tables 2 and 3). The pH of the pickle also adjusted itself to near constancy in one hour as shown (Table 2). For practical purposes a mixture of eight milliliters of buffer solution and 25 milliliters of pickle solution gave the best results.

Owing to the rapid change in the pH of the pork to near constancy, it might be assumed that the nitric oxide myohemoglobin was formed in the pork at this near constant pH and that this was the pH at which color fixation was taking place.

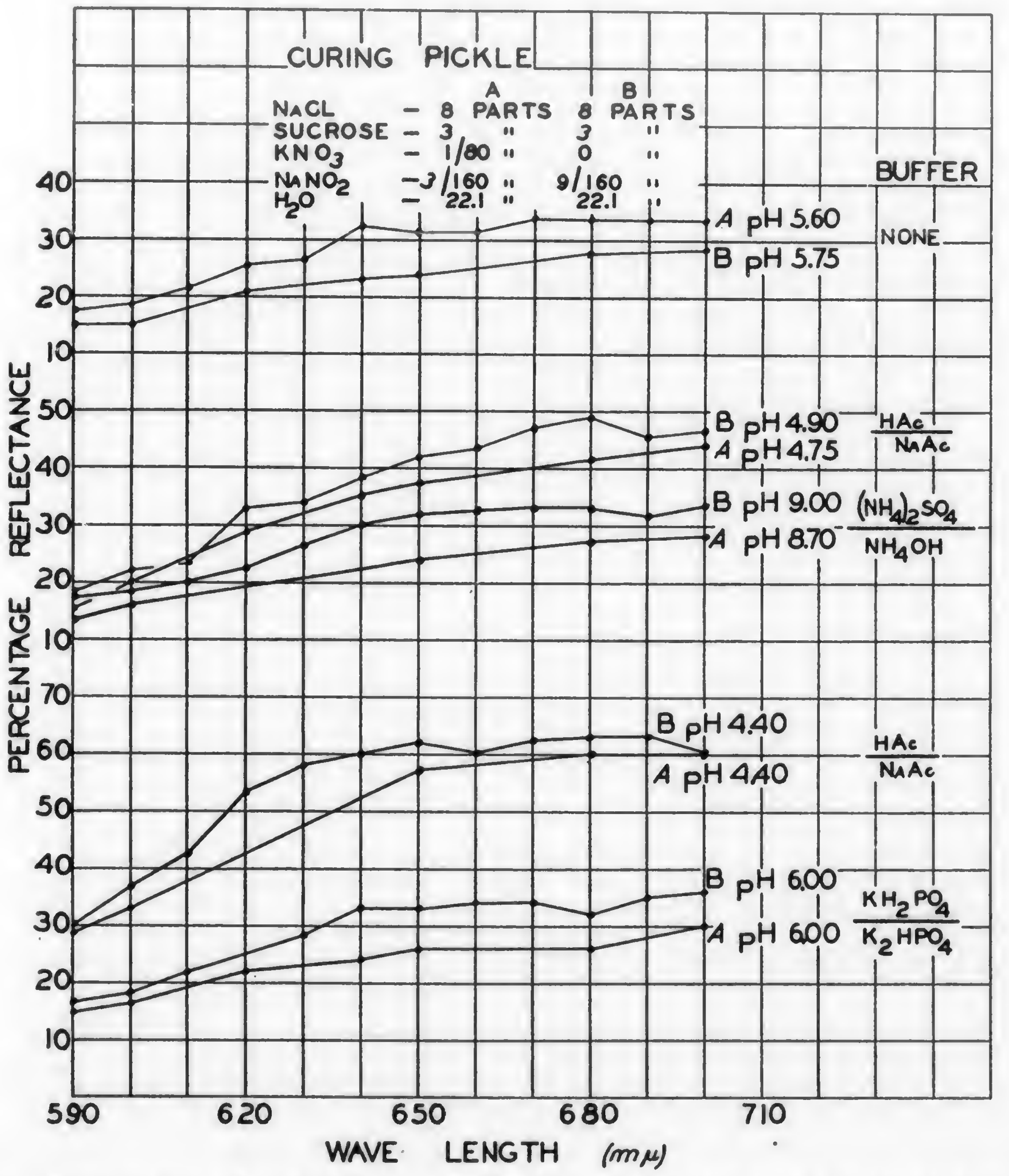


FIG. 2. Comparison of reflectance curves of pork samples cured with pickles containing different amounts of nitrite.

Samples of buffered pork cured in the described manner were cut in half and differences in color were determined as the differences in percentage reflection on the cut surface. In every case pH was also determined, and in some cases the moisture, nitrite, and chloride contents of the pork were determined in order to obtain an idea of the effect of pH on the diffusion of pickle ingredients, and in order to check the possibility of any effect on color by pH being indirectly caused by a direct effect on the curing salts. The results (Fig. 2) indicate a relationship between pH and color at a pH of below 5, resulting in insufficient color fixation between pH 4.4 and 4.9. Results (Table 3) indicate a sharp drop in the nitrite content of the cured pork below pH 5, and an apparent linear relationship between the moisture content and the pH. The pH and the chloride content of the cured pork did not appear to be related.

In order to study the effect of the nitrite content of the pickle solution on the reflectance of the pork cured at the various pH's, duplicate sets of buffered pork cubes were cured, using the described pickle solution and a pickle containing no nitrate but three times the normal amount of nitrite. The results (Fig. 2) indicate no significant color difference in the meat cured in the two pickle solutions.

TABLE 3

Effect of pH on Nitrite, Chloride, and Moisture Content of Cured Pork [1]

Buffer	Molar ratio	Moisture content of cured pork	Sodium nitrite content of cured pork	Chloride content of cured pork (dry basis)	pH after 24-hour cure		pH of original pickle
					Pickle	Pork	
		pct.	*p.p.m.*	*pct.*			
$\frac{\mathrm{HAc}}{\mathrm{NaAc}}$	32-1	59.4	1	22.7	3.70	4.40	2.75
$\frac{\mathrm{HAc}}{\mathrm{NaAc}}$	1-1	63.0	136	22.3	4.65	4.90	4.20
$\frac{\mathrm{HAc}}{\mathrm{NaAc}}$	1-32	67.3	273	23.5	5.75	5.75	5.50
$\frac{\mathrm{KH_2PO_4}}{\mathrm{K_2HPO_4}}$	32-1	64.0	375	19.8	4.85	5.50	4.00
$\frac{\mathrm{KH_2PO_4}}{\mathrm{K_2HPO_4}}$	1-1	67.3	333	23.7	6.15	6.00	5.95
$\frac{\mathrm{KH_2PO_4}}{\mathrm{K_2HPO_4}}$	1-32	66.6	375	19.0	8.15	6.45	8.90
$\frac{\mathrm{(NH_4)_2SO_4}}{\mathrm{NH_4OH}}$	32-1	67.3	375	23.1	6.00	5.70	7.60
$\frac{\mathrm{(NH_4)_2SO_4}}{\mathrm{NH_4OH}}$	1-1	69.0	200	23.6	8.50	6.50	8.80
$\frac{\mathrm{(NH_4)_2SO_4}}{\mathrm{NH_4OH}}$	1-32	69.8	188	23.2	9.25	9.00	9.95
No buffer	....	65.0	200	23.9	5.50	5.75	6.95

[1] Size of samples—20 grams, one-inch cubes; pickle solution used—25 ml. pickle solution plus eight ml. buffer solution; pH of pickle, 6.95; pH of fresh pork, 5.65; temperature, 5°C.(41°F.); control of microorganisms—30 minutes under General Electric Germicidal Lamp; salinometer reading of pickle—8.25° at 25°C.(77°F.).

Taking a wave length 700 mμ as typical, the relation between the pH and the reflectance is shown (Fig. 3).

In order to determine in a limited way the applicability of these results to large cuts of cured meat, two four-pound Boston butts were cured, one with unbuffered pickle solution and the other with phosphate buffered pickle solution. It was impractical to control microorganisms since frequent pH analyses of the pickle were made and because of the length of time

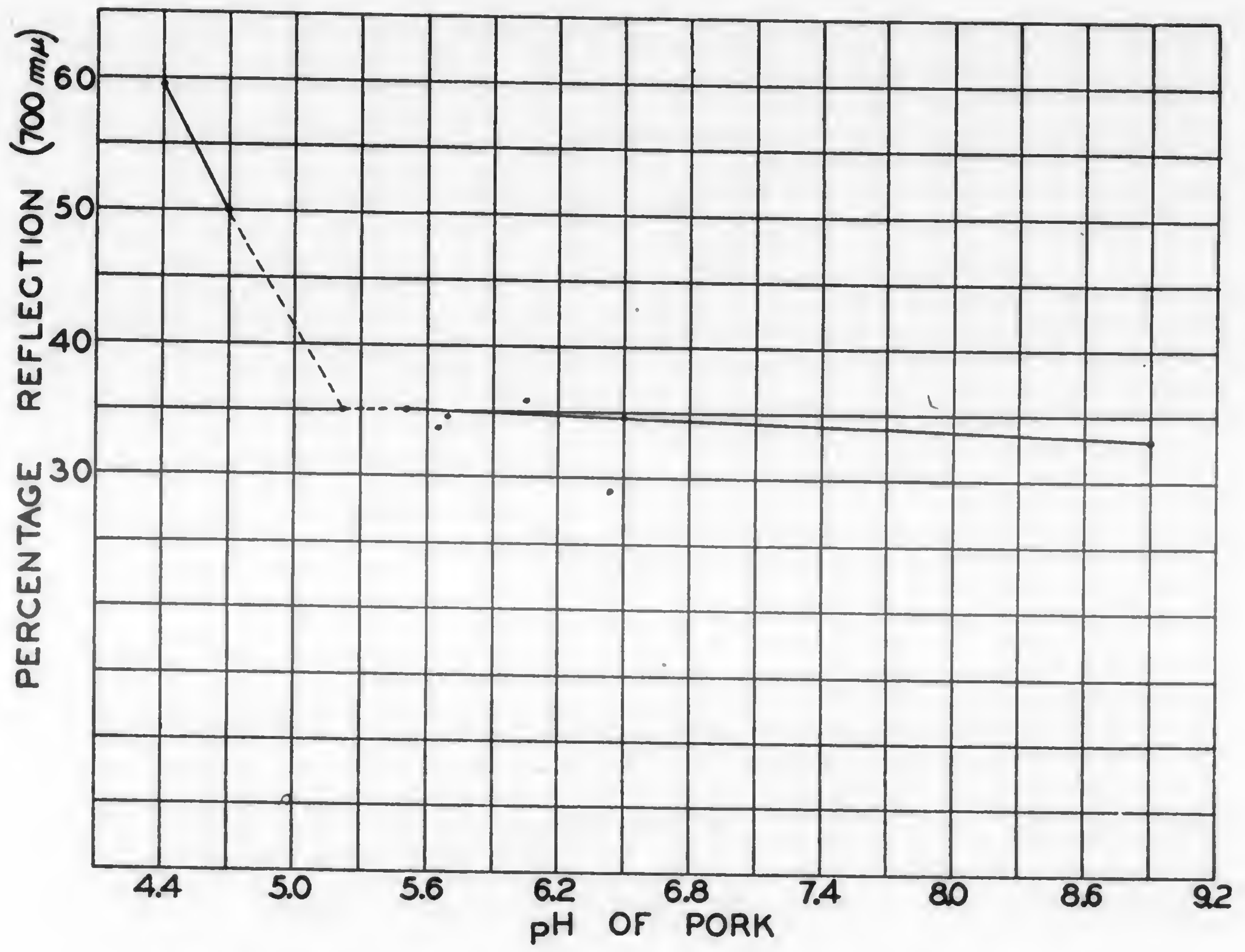


FIG. 3. Relationship between pH and reflection at 700 mμ.

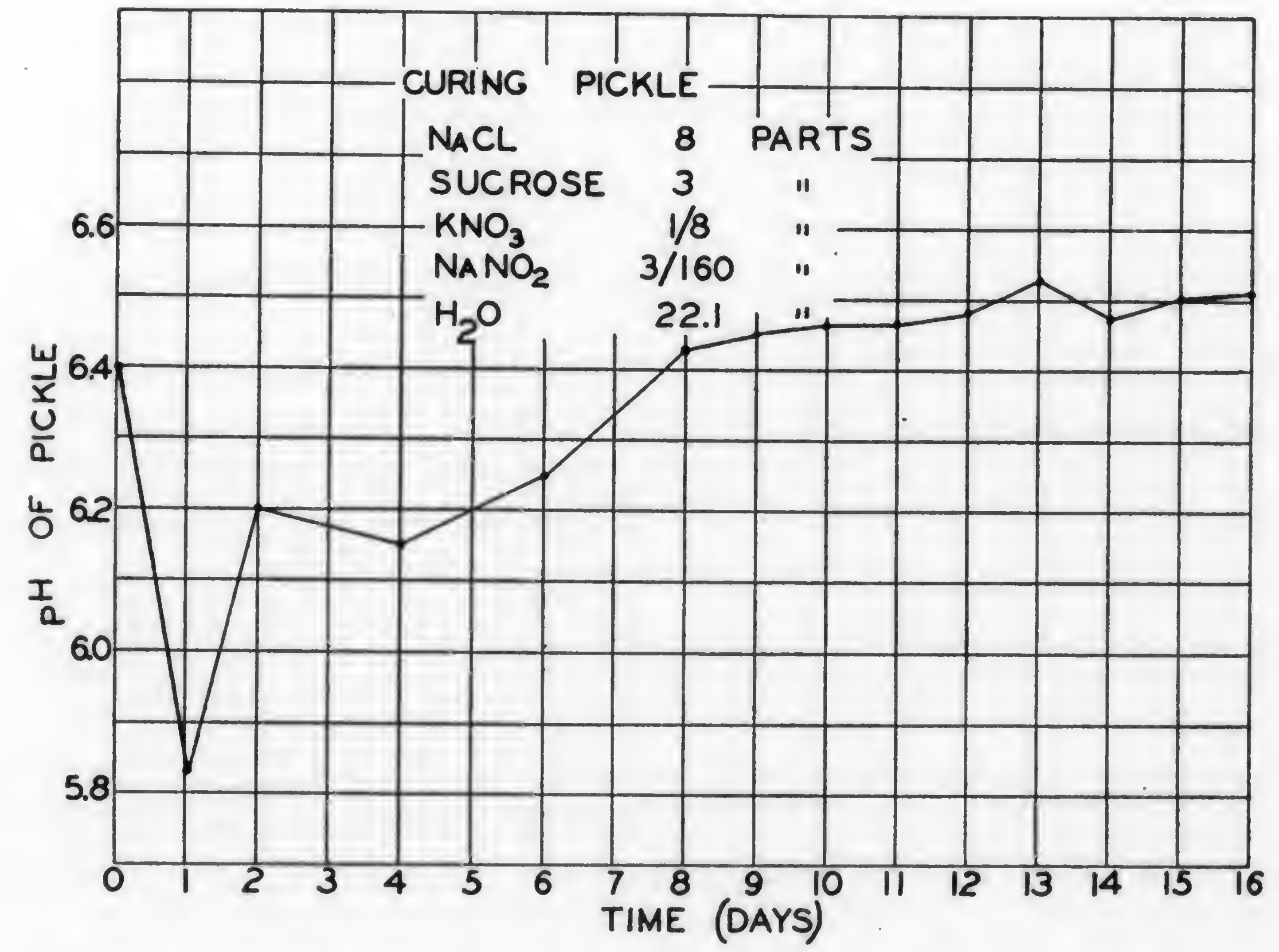


FIG. 4. Relationship between pH of pickle solution and time of curing a Boston butt.

required for the cure. The changes in pH of the pickle during curing are given (Fig. 4), and a comparison of the reflectance curves of small cured pork samples and the large cured samples (Fig. 5) indicates the two to be similar, at least over the limited pH range studied.

DISCUSSION

Owing to the high protein content of meat, it is very difficult to control its pH during cure. It is due to the buffering action of curing meat that the pH of unbuffered pickle solution falls to near that of the pork. It is probable that the pH of the pickle falls to slightly below that of the pork because of the effect of the high ionic concentration of the pickle solution on the pH of the protein buffers in the meat.

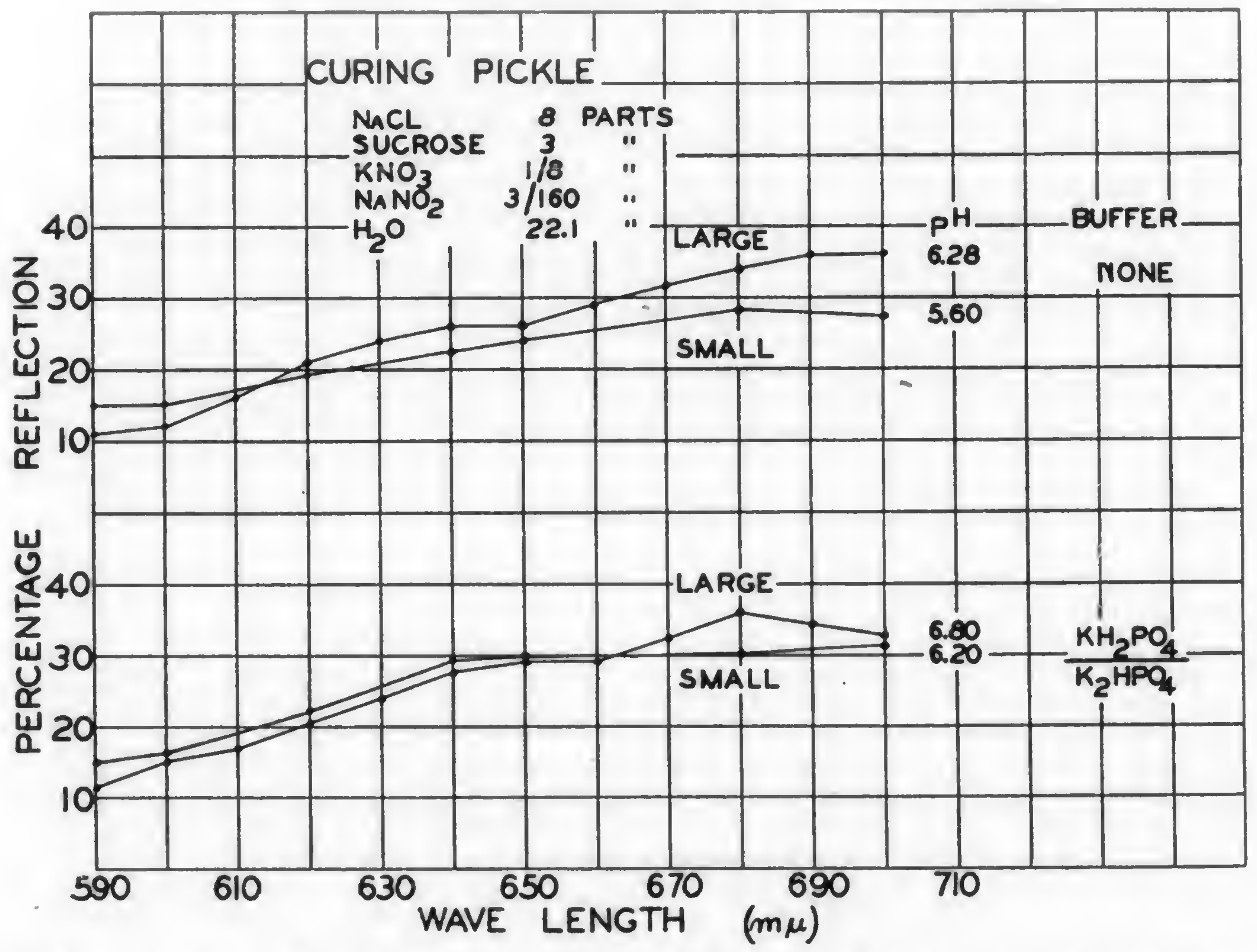


FIG. 5. Comparison of reflectance curves of large and small cured pork samples.

From the reflectance curves it is evident that above a pH of 5 there is no relationship between the pH and the color of cured meat. Between a pH of 4.4 and 4.9, color fixation is inadequate and at pH below 4 the brownish-grey color and lack of nitrite indicate no color fixation.

In the light of the chemistry of myohemoglobin and nitric oxide myohemoglobin and the results of the nitrite analysis of the cured pork, it appears that the pH plays an indirect role in the color development of cured pork.

It is well known that the reddish color of cured meat is due to the nitric oxide myohemoglobin formed by the reaction between myohemoglobin and nitrite. At a pH of 4.4 or below, the nitrite content of the meat is negligible and, therefore, there can be no color fixation.

This destruction of nitrite is due to the instability of nitrous acid which is formed in acid solution. However, at a pH of 4.9 the nitrite content of the cured meat, 136 parts per million, is many times in excess of the amount needed for proper color fixation, nevertheless color fixation is insufficient. This indicates that the myohemoglobin of the meat is in some way decolorized or destroyed at this pH. This reaction may be analogous to the conversion of oxyhemoglobin to methemoglobin by dilute acids.

From these results it is apparent that it is impossible to obtain proper color fixation below a pH of 5. It was further noted that other qualities of meat cured at a pH less than 5 were undesirable; the meat became less tender with decreasing pH, probably owing to denaturation of the meat proteins, the moisture loss or shrink increased, and the flavor became undesirable and sour.

At pH's between 5 and 6, color fixation was normal and the meat was tender and palatable. At pH's alkaline to pH 7 the color fixation was normal, but the meat became tender to point of undesirable softness in some cases. In the case of pork cured with pickle which had been made alkaline with NaOH to a pH above 8, the meat became almost gelatinous in consistency after two or three days of curing.

The similarity between the reflectance curves of the small cured samples and the Boston butts indicates that the conclusions reached regarding the small pieces are applicable to larger pieces cured for many days, at least between the pH's of 6.3 and 6.8.

In conclusion, it may be stated that small pork samples cured in the presence of sufficient nitrite at pH's above approximately 5.2 will develop a normal desirable color. A curing range between pH 5.2 and 6 would appear to give optimum results. Below pH 5 color fixation is insufficient and the meat is tough, and above pH 7.5 the meat seems to change in properties toward a gelatinous-like consistency. Curing in the range of neutrality is inadvisable owing to the low rate of reaction between nitrite and hemoglobin, Brooks (1934), and because this pH range is favorable for the growth of many proteolytic bacteria. It does not seem practicable, therefore, to enhance the color of normal cured pork by alteration of the curing pH.

The results of this investigation on small cured pork samples are in agreement with the results of Urbain and Jensen (1940) on buffered solutions of nitric oxide hemoglobin. In view of the results obtained in the present study it would appear that the results of Winkler, Hopkins, and Thistle (1940) on Wiltshire bacon might also be interpreted to confirm the findings of Urbain and Jensen (1940).

REFERENCES

Association of Official Agricultural Chemists, 1935. Official and Tentative Methods of Analysis. Fourth ed., 356-357, 506.

BROOKS, J., 1934. Changes in muscle pigments. (Gt. Brit.) Dept. Sci. and Indus. Research, Food Invest. Bd. Rpt., 35-38.

CALLOW, E. D., 1930. The scientific basis of curing. (Gt. Brit.) Dept. Sci. and Indus. Research, Food Invest. Bd. Rpt., 74-79.

DAVIES, W. L., 1932. The determination of chlorine in dairy products and biological material. Analyst 57, 79-85.

DUISBERG, P. C., 1942. The relationship of the H-ion concentration to the color developed in cured pork. Master's Thesis, Pennsylvania State College.

GÜNTHER, HANS, 1921. Über den Muskelfarbstoff. Virchows Arch. f. Path. Anat. u. Physiol. **230**, 146-176.

HAINES, R. B., 1937. Microbiology in the preservation of animal tissues. (Gt. Brit.) Dept. Sci. and Indus. Research, Food Invest. Bd. Spec. Rpt. No. 45.

HALDANE, J., 1901. The red color of salted meat. J. Hygiene **1**, 115-122.

HOAGLUND, R., 1914. Coloring matter of raw and cooked salted meats. J. Agr. Res. **3**, 211-215.

MOULTON, C. R., 1936. Effect of acidity in meat curing. Meat **5**, 10-11.

URBAIN, W. M., AND JENSEN, L. B., 1940. The heme pigments of cured meats. I. Preparation of nitric oxide hemoglobin and stability of the compound. Food Research **6**, 593-606.

WINKLER, C., HOPKINS, J. W., AND THISTLE, M. W., 1940. Canadian Wiltshire bacon. VIII. Colour of bacon and its correlation with chemical analysis. Canad. J. Res. D **18**, 217-224.

BULLETIN 440 **FEBRUARY 1943**

The Yield and Composition of Cigar-leaf Tobacco as Influenced by Fertilizer and Preceding Crop

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

Contents

PAGE
The Experimental Field 1
The Rotation 2
Field and Plot Experiments 2
Quantity and Distribution of Rainfall 4
Total annual Yields of Tobacco from the 3-year Rotation Plots for the 9-year Period 6
Yield in Relation to Method of Applying Fertilizers 8
Effect of Manure on Yield 9
Effect of Fertilizer Nitrogen on Yield and Nitrogen Content of Tobacco as Influenced by Rotation 10
Effect of Fertilizer Phosphorus on the Yield and Phosphorus Content of Tobacco as Influenced by the Rotation 12
Effect of Fertilizer Potash on Yield and Potassium Content of Tobacco as Influenced by Rotation 13
Nitrogen and Potassium Content and Total Yield of Cigar-leaf Tobacco as Influenced by Fertilizer Treatment with Corn as the Preceding Crop 15
Burn and Quality of Cigar-leaf Tobacco as Influenced by Fertilizer Treatment and Preceding Crop 16
Discussion 19
Summary 21
Bibliography 21

Acknowledgments

The authors gratefully acknowledge the co-operation and assistance of Bayuk Cigars, Inc., of Philadelphia, Pa., and The American Potash Institute of Washington, D. C.

The Yield and Composition of Cigar-leaf Tobacco as Influenced by Fertilizer and Preceding Crop[*]

D. E. HALEY[†], O. E. STREET[‡], M. A. FARRELL[§] and J. J. REID[°]

CONSIDERABLE TIME AND ATTENTION have been given to studies relating to the development and improvement of the cigar-leaf tobacco industry of Pennsylvania which is centered in Lancaster county. Investigational work on this particular crop was initiated by The Pennsylvania State College and the Lancaster County Tobacco Growers Association in 1892. Since 1912, this program in both field and laboratory has benefited materially through close co-operation with the Bureau of Plant Industry of the United States Department of Agriculture.

In 1931 plans were prepared for a series of field experiments to be undertaken which would feature principally a 3-year rotation system of wheat, clover, and tobacco, with the tobacco receiving different fertilizer treatments, with or without supplemental additions of manure. It was hoped that these experiments which were to continue for a period of nine years would result in beneficial effects on the fertility of the soil which would be reflected in the quality and yields of tobacco produced. Other field experiments, formerly conducted at Ephrata, were discontinued immediately, and a new experimental field was selected for this undertaking near Roseville, just north of the city of Lancaster.

THE EXPERIMENTAL FIELD

The experimental field having an area of 10 acres has been described in a previous publication *(3)*. The soil, principally Hagerstown Silt Loam, was found to be definitely alkaline in reaction, although it had received no lime treatment since 1910. No fertilizers had been used on the field for several years before the experiments were started in 1932, and the field had remained in volunteer growth since 1929.

The greater part of the field was set aside for the 3-year rotation experiment and the specific area was divided into 3 tiers of 31 plots, each of which was one-twenty-first of an acre in area. The

---

[*] Authorized for publication January 15, 1943. This investigation was conducted jointly by the Departments of Agricultural and Biological Chemistry and of Bacteriology of the Pennsylvania Agricultural Experiment Station and the Division of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

[†] Professor of Soil and Phytochemistry.

[‡] Agronomist, Division of Tobacco Investigations, U.S.D.A. Before 1939 the conduct of the field work was delegated to the late Otto Olson. After that time, this junior author was in charge.

[§] Professor and Head of Bacteriology.

[°] Associate Professor of Bacteriology.

Fig. 1.—Harvesting Pennsylvania cigar-leaf tobacco. Note that the planting was made on the contour of the field to forestall erosion.
(Photo by Soil Conservation Service)

remainder of the 10-acre tract was devoted to other field experiments.

THE ROTATION

In 1932 tobacco was grown on tier I following weeds. Wheat was grown on tier III the same year. Legumes were grown on tier II the fall of 1932 and a heavy crop was plowed under on this particular tier the latter part of May, 1933.

Once the experiment was under way, the three crops involved were grown simultaneously each season. Clover preceded tobacco on all plots with the exception of 29, 30, and 31. On these plots alfalfa preceded tobacco.

FIELD AND PLOT TREATMENTS

When wheat first appeared in the rotation the entire tier received a uniform application of 300 pounds of superphosphate (16 per cent P_2O_5) per acre. No supplemental treatments were given the legumes, but the plots devoted to tobacco on a given tier received individual fertilizer treatments as listed in table 1. Whenever manure was used, it was applied approximately one month before transplanting the seedlings. Other additions, with the exception of nitrate of soda, which was applied as a side-dressing one month following transplanting, were applied broadcast or in the row approximately one week before transplanting. It may be stated, however, that side-dressing with nitrate of soda was discontinued in 1938, after which it was applied broadcast.

Table 1.—Field treatments of tobacco experimental plots, tiers I, II and III, of the 3-year rotation system; all quantities on the basis of one acre; Roseville, 1932-40.

PLOT No.	MANURE	FERTILIZER TREATMENT QUANTITY	FERTILIZER TREATMENT FORMULA	LIME	FERTILIZER APPLICATION
	tons	*pounds*		*pounds*	
1	—	1,000	6-8- 4	——	Broadcast
2	—	1,000	6-8- 8	——	”
3	—	1,000	6-8-12	——	”
4	—	1,000	6-8-16	——	”
5*	—	1,000	0-8-12	——	”
6	—	1,000	3-8-12	——	”
7	—	1,000	9-8-12	——	”
8	—	1,000	6-0-12	——	”
9	—	1,000	6-4-12	——	”
10*	—	1,000	0-8-12	——	”
11	—	500	6-8-12	——	”
12	—	1,500	6-8-12	——	”
13	—	1,000	6-8-12	1,000	”
14	—	1,000	6-8-12	——	”
15*	—	1,000	0-8-12	——	”
16	—	500	6-8-12	——	In row
17	—	1,000	6-8-12	——	”
18	—	1,500	6-8-12	——	”
19	—	——	——	——	——
20*	—	1,000	0-8-12	——	Broadcast
21	10	1,000	0-8-12	——	”
22	10	1,000	3-8-12	——	”
23	10	1,000	3-8- 8	——	”
24	10	500	3-8-12	——	”
25*	—	1,000	0-8-12	——	”
26	10	1,000	3-8-16	——	”
27	10	——	——	——	——
28	20	——	——	——	——
29†	—	——	——	——	——
30*†	—	1,000	0-8-12	——	Broadcast
31†	—	1,000	6-8-12	——	”

* Replicates.
*† Alfalfa in rotation instead of clover.

Superphosphate (16 per cent P_2O_5), was used as a carrier of phosphorus, while sulfate of potash was used as a carrier of potassium in all cases.

Cottonseed meal and nitrate of soda constituted the nitrogen carriers. During the first 6-year period two-thirds of the total nitrogen was supplied in the form of cottonseed meal and one-third as nitrate of soda, with the exception of plot 14 where these conditions were reversed. During the last 3-year period two-thirds of the nitrogen was applied as nitrate of soda and one-third as cottonseed meal.

Plot 13 received an application of 1000 pounds of hydrated lime per acre when tobacco was grown, but was the only plot of a given tier to receive this supplemental treatment.

QUANTITY AND DISTRIBUTION OF RAINFALL

The quantity and distribution of rainfall throughout the growing season appears to be of overshadowing importance in the production of Pennsylvania cigar-leaf tobacco. It should be ample, not excessive, and at the same time should be well distributed to produce best results. While the rainfall during the growing season of 1936 was slightly less than normal, it was well distributed. As a result, a high-yielding crop free of leafspots was obtained that season.

An interruption of growth due to lack of sufficient rainfall may prove deleterious from the standpoint of both yield and leafspot disorders, especially if the drought is broken abruptly. However, these effects appear to be controlled in large measure by the growth stage of the plant or what might be termed its physiological age. It is a matter of common knowledge that a young plant is less susceptible to the effects of a drought and the breaking of the same than is true for a plant approaching maturity.

The weekly rainfall for six months of every season during the 9-year period covered by the tests herein reported are presented in table 2. The records for the fourth week of every month include the additional days within the month.

A striking example of excessive and poorly distributed rainfall and the incidence of leafspots is reported by Olson *(1)* for 1933. A heavy stand of red clover was plowed under on May 24 of that year; and from that time until the tobacco seedlings were transplanted on June 3, the rainfall totaled but 1.31 inches. During the remainder of the month the rainfall totaled 1.34 inches. From July 1 to 25 the rainfall measured 3.7 inches. Practically all of this, however, fell in two 24-hour periods, on July 4 and 16. The heavy rainfall of the 4th was followed by 12 days of hot dry weather and the rain of the 16th by 10 days of hot weather with negligible precipitation. This condition of drought was broken abruptly on July 26 when the rainfall amounted to 4.9 inches. Following another week of hot weather, the packed and baked soil was subjected to another beating rain amounting to 4.2 inches on August 3 with disastrous results. According to Olson *(1)*, the danger from wildfire became so apparent that the tobacco from all the plots of the 3-year rotation experiment was harvested in an immature condition to prevent total loss.

In 1937 conditions approached those of 1933. Following a heavy downpour of 2.35 inches on July 6, precipitation was negligible until the last week of the month when the period of drought was broken with heavy, beating rains. Another period of dry weather was broken abruptly with heavy rains during the second week of August and leafspot damage became severe. It is of interest to note, however, that two rows of each plot were harvested on August 19 and the yields were calculated from these. The remaining plants being subjected to a rainfall on August 21 and 22 of 4.65 inches were practically destroyed by leafspots. However, where tobacco followed corn on another part of the experimental field and where relatively large quantities of potash were used, the results were much better. Regarding these observations, Olson *(2)* made the following statement: "Apart from the recognized fact that heavy applications of potash are necessary for 'quality' in cigar-filler tobacco, it is gradually being noted that potash renders invaluable aid in checking the ravages of that virulent type of leafspot commonly known as 'wildfire.' Cigar-filler tobacco in Pennsylvania is sorted into two grades: 'fillers' or low grade tobacco, and 'wrappers' which represents the best grade. In normal years the ratio is about one-fourth fillers to three-fourths wrappers, but in years of high wildfire infection the ratio may be reversed, in spite of practical sanitary precautions for wildfire prevention. Following corn the experimental plots fertilized with

Table 2.—Weekly rainfall recorded for months of March to September; Roseville, 1932-40.

MONTH	WEEK	1932	1933	1934	1935	1936	1937	1938	1939	1940	NORMAL
		inches	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*
March	1st	1.52	0.25	0.08	0.23	0.00	0.10	0.10	0.50	1.52	
	2nd	0.00	1.59	0.00	0.87	2.10	0.25	1.65	0.72	1.53	
	3rd	0.87	2.96	0.00	0.10	1.82	0.50	0.55	0.00	0.00	
	4th	2.76	0.53	2.04	0.55	0.75	0.25	0.05	1.00	0.50	
	TOTAL	5.15	5.33	2.12	1.75	4.67	1.10	2.35	2.22	3.55	3.53
April	1st	0.09	1.55	0.32	0.52	1.40	0.55	0.05	1.20	0.71	
	2nd	1.37	1.28	0.29	2.78	0.95	0.20	0.65	0.60	3.16	
	3rd	0.00	2.21	0.09	0.20	0.15	1.60	0.70	1.20	2.16	
	4th	0.33	0.74	0.15	0.50	0.00	1.72	0.40	0.40	0.00	
	TOTAL	1.79	5.78	0.85	4.00	2.50	3.07	1.80	3.40	6.03	3.55
May	1st	3.17	0.99	1.23	0.92	0.70	0.10	0.32	0.00	0.35	
	2nd	0.00	1.71	0.05	0.48	0.20	1.20	0.94	0.25	0.38	
	3rd	0.00	0.78	0.21	0.10	0.42	0.15	0.77	0.10	1.21	
	4th	0.00	1.35	1.24	0.00	0.15	1.20	0.99	0.00	2.46	
	TOTAL	3.17	4.83	2.73	1.50	1.47	2.65	3.02	0.35	4.40	3.53
June	1st	0.00	0.21	0.90	0.56	0.18	0.35	0.92	0.20	0.86	
	2nd	0.45	1.10	1.37	0.65	1.90	0.60	2.10	1.35	0.25	
	3rd	2.62	0.03	0.62	0.62	1.00	2.95	0.00	0.75	0.60	
	4th	2.20	0.01	0.60	0.78	1.17	1.40	1.95	1.19	1.50	
	TOTAL	5.27	1.55	3.49	2.61	4.25	5.30	4.97	3.49	3.21	4.22
July	1st	1.00	1.90	0.35	0.00	1.62	2.85	0.11	0.23	0.82	
	2nd	0.00	0.20	2.30	2.10	0.10	0.50	1.58	1.02	0.31	
	3rd	0.40	1.50	0.62	1.23	0.25	0.10	1.65	0.00	0.22	
	4th	1.60	5.20	1.01	1.00	1.75	4.65	1.91	0.88	0.57	
	TOTAL	3.00	8.80	4.28	4.33	3.72	8.10	5.25	2.13	1.92	4.54
Aug.	1st	2.20	4.20	0.00	0.30	0.90	0.90	0.32	0.55	0.33	
	2nd	0.30	1.17	1.80	0.45	0.40	3.48	0.78	0.93	0.68	
	3rd	0.70	2.44	0.78	0.48	2.00	2.45	0.42	2.88	0.39	
	4th	1.00	5.13	0.64	0.67	1.15	3.60	0.70	0.50	3.39	
	TOTAL	4.20	12.94	3.22	1.90	4.45	10.43	3.33	4.86	4.69	4.90
Sept.	1st	0.60	1.99	0.30	3.30	0.20	0.40	0.54	1.19	0.00	
	2nd	0.00	1.10	2.34	0.25	0.10	0.15	0.20	0.49	0.19	
	3rd	0.00	0.02	1.85	0.25	0.30	0.15	2.40	0.02	0.15	
	4th	0.70	0.00	3.90	0.00	1.50	0.30	0.10	0.43	4.66	
	TOTAL	1.30	3.11	8.39	3.80	2.10	1.00	3.24	2.13	5.01	

from 240 to 320 pounds of sulfate of potash per acre in addition to nitrogen and phosphoric acid, the ratio was one-third fillers to two-thirds wrappers in 1933 and 1937."

The gradual breaking of a drought without serious effects was noted in 1940. On August 20 of that season, a survey of the plots in the 3-year rotation indicated a condition of drought. The rainfall in July amounted to 1.92 inches, and from August 1 to 25 but 1.30 inches were recorded. On August 26 the drought was broken with 0.83 inch of rainfall, followed by a day of practically no rainfall. Another 0.80 inch fell on the 28th, 0.10 inch on the 29th and 0.36 inch on the 30th. This was followed by 1.27 inches on the 31st, a total of 3.39 inches for the 5-day period. Following this, only 0.23 inch fell until the crop was harvested on September 17. This gradual shift from a drought to a moderate moisture supply was not accompanied by the ill effects noted in the years of sudden and excessive transition.

TOTAL ANNUAL YIELDS OF TOBACCO FROM THE 3-YEAR ROTATION PLOTS FOR THE 9-YEAR PERIOD

The yields of tobacco obtained from the individual plots of the 3-year rotation experiment from 1932 to 1940 inclusive are presented in table 3. The rank of the plots according to yield arranged in descending order may be found in table 4.

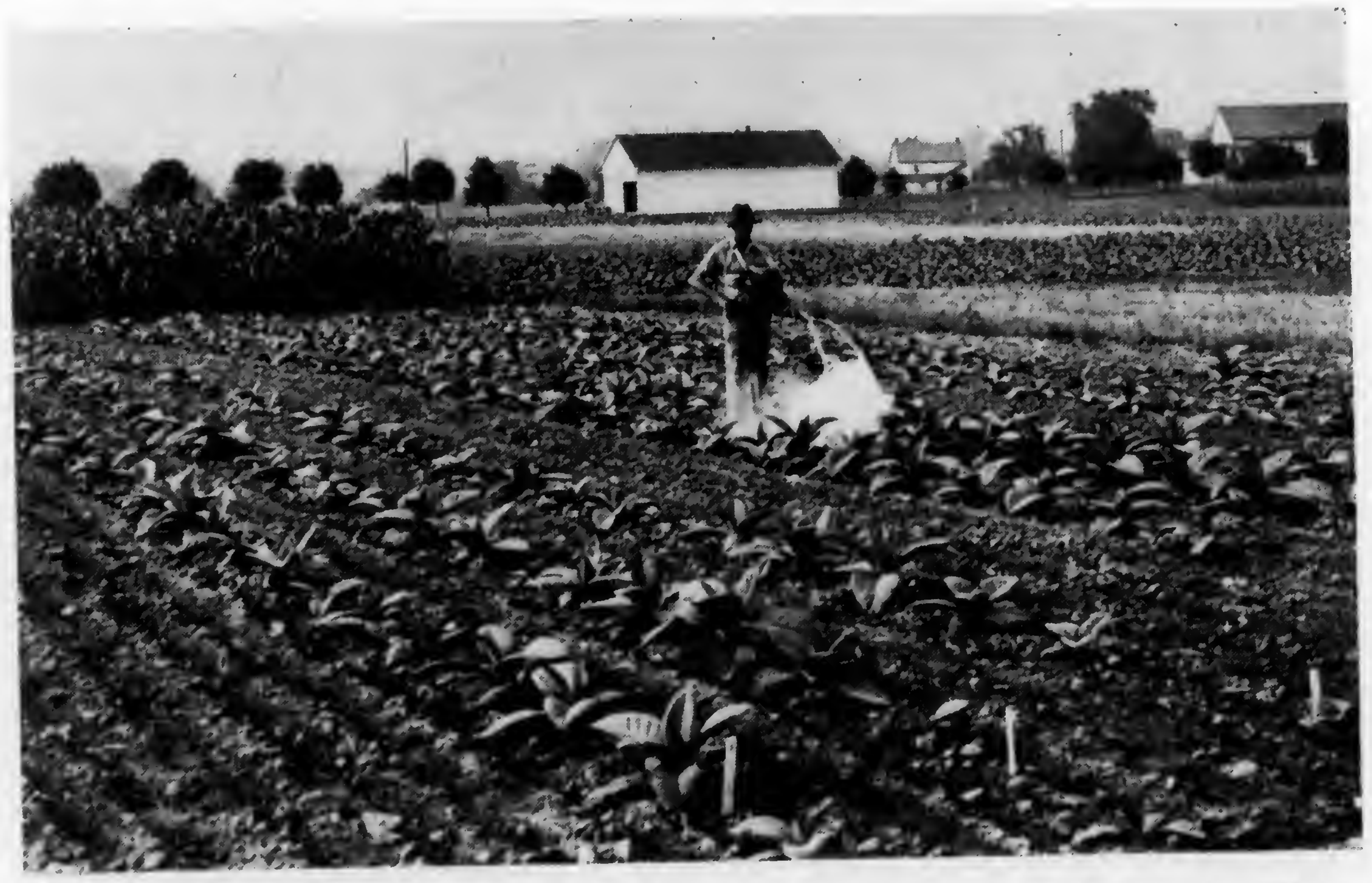


Fig. 2.—Cigar-leaf tobacco must be dusted to control insects. This picture also presents a general view of the first experimental plots at Roseville, Pa. The building in the background is the experimental curing shed.

Table 3.—Total annual yields in pounds of tobacco per acre obtained from plots of the 3-year rotation experiment; Roseville, 1932-40.

PLOT										AVERAGE ANNUAL YIELD		
No.	1932	1933	1934	1935	1936	1937	1938	1939	1940	'32-34	'35-40	'32-40
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
1	1783	1218	1743	1995	1995	1491	1660	1325	1729	1581	1699	1660
2	1911	1365	1890	2100	2037	1596	1368	1430	1997	1722	1755	1744
3	1925	1407	1964	2163	2058	1659	1505	1448	1711	1765	1757	1760
4	1827	1099	1617	1995	1890	1617	1584	1362	1725	1514	1696	1635
5	1281	1070	1502	1470	1617	1260	1136	1101	1594	1284	1363	1337
6	1596	1218	1386	1722	1890	1491	1279	1290	1526	1400	1533	1489
7	1596	1218	1533	1953	2016	1522	1430	1457	1639	1449	1670	1596
8	1554	966	1313	1785	1895	1428	1224	1331	1562	1278	1538	1451
9	1638	1155	1481	1764	1785	1365	1342	1424	1596	1425	1546	1506
10	1512	1008	1449	1607	1617	966	1232	1305	1675	1323	1400	1375
11	1734	1050	1470	1701	1795	1155	1594	1365	1651	1418	1544	1502
12	1806	1281	1670	1932	1932	1533	1729	1332	1731	1586	1698	1661
13	1638	1134	1817	1764	1848	1512	1373	1284	1659	1530	1573	1559
14	1743	1134	1743	1911	1837	1449	1464	1307	1703	1540	1612	1588
15	1554	1134	1418	1523	1638	1323	1240	1282	1589	1369	1433	1411
16	1869	1239	1691	1764	1774	1428	1236	1366	1594	1600	1527	1551
17	2352	1386	1974	1985	2079	1533	1523	1473	1763	1904	1726	1785
18	2583	1512	2048	2142	2289	1722	1691	1352	1817	2048	1836	1906
19	1050	987	1355	1208	1512	1092	953	1044	1435	1131	1207	1182
20	1680	1155	1701	1617	1638	1281	1263	1238	1621	1512	1443	1466
21	1764	1260	1869	1869	1743	1407	1440	1306	1666	1631	1572	1592
22	2205	1428	1895	2100	1995	1680	1600	1282	1792	1843	1742	1775
23	2058	1269	1869	1806	1724	1449	1379	1236	1816	1729	1568	1622
24	1869	1260	1764	1848	1700	1449	1382	1273	1926	1831	1596	1608
25	1680	1260	1901	1911	1659	1281	1262	1316	1836	1614	1544	1567
26	2163	1134	1869	1932	1848	1764	1648	1382	2147	1722	1787	1765
27	1869	1260	1691	1869	1837	1470	1458	1259	1948	1607	1640	1629
28	1932	1407	2037	1974	2089	1743	1676	1312	2143	1792	1823	1813
29	1155	966	1439	1565	1659	1176	1476	1235	1747	1187	1476	1380
30	1659	1134	1712	1932	1995	1654	1374	1367	2019	1502	1724	1650
31	1785	1260	2129	2027	2247	1701	1312	1447	1976	1725	1785	1765
Av.	1767	1205	1708	1837	1859	1458	1414	1320	1753	1560	1607	1591

The first 3-year period did not necessarily represent the rotation, as the system did not become established until tobacco appeared on tier I in 1935. Moreover, it should be remembered that the 1933 crop, and to a certain extent that of 1937, was harvested in an immature condition, which affected not only yield but chemical composition as well.

Plot 3, the highest-yielding plot in 1935, was seventeenth in 1940, while plot 17, the highest ranking plot for 1939, was twelfth in 1940. It would appear, therefore, that in addition to moisture conditions, the plowing under of old legume residues interfered seriously with the response of tobacco to a given fertilizer treatment.

The yields obtained over the 6-year period, 1935-40, were highly inconsistent in the majority of cases. For example, plot 28, the highest ranking plot of 31 from the standpoint of yield during this period, was seventeenth in 1939. Moreover, plot 18, second highest in yield for the period, was twelfth in rank for 1939.

Table 4.—Rank in descending order of plot treatments with respect to annual yields of tobacco in pounds per acre, on the 3-year rotation experiment; Roseville, 1932-40.

										AVERAGE		
RANK	1932	1933	1934	1935	1936	1937	1938	1939	1940	'32-34	'35-40	'32-40
1st	18	18	31	3	18	26	12	17	26	18	18	18
2nd	17	22	18	18	31	28	18	7	28	17	28	28
3rd	22	28	28	2	28	18	28	3	30	22	26	17
4th	26	3	17	22	17	31	1	31	2	28	31	22
5th	23	17	3	31	3	22	26	2	31	3	3	26
6th	28	2	22	1	2	3	22	9	27	23	2	31
7th	3	12	25	4	7	30	11	26	24	31	22	3
8th	2	21	2	17	22	20	4	30	25	2	17	2
9th	24	23	23	28	30	4	17	16	18	26	30	12
10th	16	24	26	7	1	2	3	11	23	21	1	1
11th	27	25	21	12	12	17	29	4	22	24	12	30
12th	4	27	13	30	4	12	14	18	17	25	4	4
13th	12	31	24	26	6	7	27	12	29	27	7	27
14th	31	16	1	14	8	13	21	8	12	16	27	23
15th	1	6	14	25	26	1	7	1	1	12	14	24
16th	21	7	30	27	13	6	24	25	4	1	24	7
17th	14	1	20	21	14	27	23	28	3	14	13	21
18th	11	20	27	24	27	24	30	14	14	13	21	14
19th	25	9	16	23	11	23	13	21	10	4	23	25
20th	20	13	12	8	9	14	2	10	21	20	9	13
21st	30	14	4	13	16	8	9	6	13	30	11	16
22nd	13	15	7	16	21	16	31	13	11	7	25	9
23rd	9	26	5	9	23	21	6	22	7	9	8	11
24th	6	30	9	6	24	9	20	15	20	11	6	6
25th	7	4	11	11	29	15	25	24	9	6	16	20
26th	8	5	10	20	25	25	15	27	5	15	29	8
27th	15	11	29	10	20	5	16	20	16	10	20	15
28th	10	10	15	29	15	29	10	23	15	5	15	29
29th	5	19	6	15	5	11	8	29	8	8	10	10
30th	29	8	19	5	10	19	5	5	6	29	5	5
31st	19	29	8	19	19	10	19	19	19	19	19	19

YIELD IN RELATION TO METHOD OF APPLYING FERTILIZERS

It may be noted that plots 16, 17, and 18 of the 3-year rotation experiment received varying quantities of fertilizers "in the row." In contrast, plots 11, 3, and 12 received the same quantities and kind of fertilizers corresponding to plots 16, 17, and 18, respectively, but these were applied "broadcast." The results obtained from broadcasting or applying fertilizers in the row are presented in table 5. While little difference existed between the treatments with 500 and 1000 pounds of fertilizer per acre, the application of 1500 pounds in the row was superior to that of broadcasting.

Table 5.—A comparison of row treatment with broadcasting of fertilizers as indicated by yields of tobacco at Roseville.

	FERTILIZER TREATMENT				RANK IN YIELD FOR THE SIX-YEAR PERIOD		
PLOT No.	Quantity	Formula	Method of Application	AVERAGE	Lowest	Highest	Average
	pounds per acre			*pounds per acre*			
11	500	6-8-12	Broadcast	1544	29th	7th	21st
16	500	6-8-12	Row	1527	27th	9th	25th
3	1000	6-8-12	Broadcast	1757	17th	1st	5th
17	1000	6-8-12	Row	1726	12th	1st	8th
12	1500	6-8-12	Broadcast	1698	14th	1st	11th
18	1500	6-8-12	Row	1836	12th	1st	2nd

EFFECT OF MANURE ON YIELD

The soil on which the 3-year rotation experiment was conducted was rather heavy. This is more or less characteristic of the Lancaster county tobacco soils in general. To partially alleviate this condition by improving the structure, fresh manure was used in quantities varying from 10 tons to 20 tons per acre. Such applications were made with and without supplemental fertilizer applications in the 3-year rotation experiment.

The results obtained in regard to the effect of manure on yields are listed in table 6. These results show that while the 3-year rotation experiment was in operation, plot 28, receiving an application of manure alone, at the rate of 20 tons per acre, was second from the standpoint of yield. However, in 1939, a relatively dry season, it was seventeenth in rank of yield. Plot 27, receiving manure alone, at the rate of 10 tons per acre, averaged fourteenth in rank of yield for the 6-year period, but in 1939 was twenty-sixth.

Plot 26, receiving 10 tons of manure and 1000 pounds of a 3-8-16 mixture per acre, was first in yield in 1940 and also in 1937, the year that leafspots were so prevalent. This particular plot averaged third from the standpoint of yield over the 6-year period.

Table 6.—Effect of manure when used alone or with supplemental fertilizer treatments on the yield of tobacco from plots of the 3-year rotation experiments; Roseville, 1935-40.

PLOT No.	MANURE PER ACRE	FERTILIZER TREATMENT		AVERAGE YIELDS 1935 to 1940	RANK OF YIELD 1935 TO 1940		
		Quantity	Formula		Lowest	Highest	Average
	tons per acre	*pounds per acre*		*pounds per acre*			
19	(Check)	——	——	1,207	31st	30th	31st
27	10	——	——	1,640	26th	6th	14th
21	10	1,000	0-8-12	1,572	23rd	14th	18th
25	None	1,000	0-8-12	1,544	26th	8th	22nd
24	10	500	3-8-12	1,596	25th	7th	16th
23	10	1,000	3-8- 8	1,568	28th	10th	19th
22	10	1,000	3-8-12	1,742	23rd	4th	7th
26	10	1,000	3-8-16	1,787	15th	1st	3rd
28	20	——	——	1,823	17th	2nd	2nd

The next ranking plot of the manure series from the standpoint of yield is plot 22, receiving 10 tons of manure and 1000 pounds per acre of a 3-8-12 mixture. The yield of this plot was definitely superior to plot 24 which received 10 tons of manure and 500 pounds per acre of a 3-8-12 mixture.

Fresh manure during a dry season may have injurious effects on the growth of tobacco. Soil organisms, which are instrumental in bringing about its decay, may seriously compete with tobacco for nutrients. Moreover, the manure may compete with tobacco for moisture which may be present. Fundamentally, the use of well rotted or partially rotted manure is to be recommended, as there is less danger from the competition of soil organisms for nutrients, and, moreover, the manure being humified should act as a reservoir of moisture for the growing plants.

EFFECT OF FERTILIZER NITROGEN ON YIELD AND NITROGEN CONTENT OF TOBACCO AS INFLUENCED BY ROTATION

Plots 3, 5, 6, and 7 received varying quantites of nitrogen in the fertilizers used, while the concentrations of both phosphorus and potassium were held constant. The results obtained are presented in table 7.

Table 7.—Yields of tobacco as influenced by varying quantities of nitrogen applied in fertilizer; Roseville, 1935-40.

PLOT No.	FERTILIZER TREATMENT		AVERAGE YIELD 1935 to 1940	RANK OF YIELD 1935 TO 1940		
	Quantity	Formula		Lowest	Highest	Average
	pounds per acre		*pounds per acre*			
5	1,000	0-8-12	1,363	30th	26th	30th
6	1,000	3-8-12	1,533	30th	13th	24th
3	1,000	6-8-12	1,757	17th	1st	5th
7	1,000	9-8-12	1,670	23rd	2nd	13th

From the results presented in table 7, it is apparent that an application of 60 pounds of nitrogen per acre, plot 3, had a desirable influence on yield. However, the results over the 9-year period showed marked inconsistencies which may be attributed to the overshadowing influence of the particular rotation.

While the nitrogen supplied should have aided in the bacterial degradation of the old legume residues and therefore favor growth, it appears that the total nitrogen supplied and that inherently present in the soil was not necessarily reflected in the quantity of nitrogen found in the leaves of the mature plants.

Analyses were made of samples of mature tobacco taken from all plots during the season of 1935 to 1939, inclusive. The results obtained are listed in table 8.

It is the general opinion that for best results, ample quantities of nitrogen in the proper form should be at the disposal of the tobacco plant during its period of active growth. Growth interruptions due to a lack of nitrogen are harmful, especially when followed by relatively large quantities made available at a state of growth where it cannot be metabolized efficiently.

It may be noted that the nitrogen content of tobacco from all plots averaged but 3.23 per cent, although cigar-leaf tobacco is

Table 8.—Nitrogen content of mature tobacco (sixth leaf) grown on the 3-year rotation plots over a period of five years, on a moisture-free basis; Roseville, 1935-39.

PLOT No.	1935	1936	1937	1938	1939	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	3.95	3.71	3.36	3.32	3.87	3.64
2	3.72	4.41	3.00	2.78	3.75	3.43
3	3.32	3.90	3.49	2.64	4.14	3.50
4	3.31	3.70	2.84	2.76	4.12	3.35
5	2.94	3.13	2.85	2.62	3.78	3.06
6	3.50	3.67	2.98	2.42	3.80	3.27
7	3.77	3.52	3.21	2.83	3.86	3.44
8	3.54	4.11	3.54	3.03	4.00	3.64
9	3.66	3.19	3.26	3.00	4.31	3.48
10	2.72	3.57	2.64	2.49	3.69	3.02
11	3.52	3.32	3.10	3.04	3.96	3.39
12	3.77	3.92	2.54	3.05	4.11	3.48
13	3.44	3.45	2.82	2.61	3.99	3.26
14	3.40	3.83	2.98	2.66	3.88	3.35
15	2.26	3.24	2.50	2.54	3.59	2.83
16	2.97	3.14	2.64	2.72	4.19	3.13
17	3.04	3.65	2.54	3.20	3.90	3.27
18	3.45	3.32	3.06	3.46	3.92	3.44
19	3.46	2.92	2.85	2.97	3.56	3.15
20	2.67	3.16	2.55	2.34	3.76	2.90
21	2.60	2.95	2.14	2.71	3.62	2.80
22	3.02	3.65	2.42	2.78	3.74	3.12
23	3.16	3.34	2.47	2.89	3.87	3.15
24	3.31	3.78	2.67	2.79	3.78	3.27
25	2.61	3.06	2.19	2.53	3.51	2.78
26	3.26	3.28	2.37	2.81	3.72	3.09
27	3.00	2.85	2.36	2.71	3.89	2.96
28	3.65	3.74	2.84	2.80	3.80	3.37
29	2.62	3.86	2.56	3.19	4.05	3.26
30	2.52	3.68	2.32	2.74	3.51	2.95
31	2.93	4.16	3.09	2.81	3.34	3.27
AVERAGE	3.20	3.52	2.78	2.81	3.84	3.23

looked upon as a high-nitrogen crop. It is interesting to note that the highest percentage of nitrogen in the leaf for the 5-year period was from plot 1, a low potassium plot, and plot 8, a no-phosphorus plot. While plots receiving no direct application of nitrogen-carrying materials yielded tobacco which was usually low in respect to this element, it is interesting to note that plot 5, a 0-8-12 plot, averaged a nitrogen content of 3.06 per cent, while plot 27, receiving 10 tons of manure, yielded tobacco averaging but 2.96 per cent.

The lowest nitrogen content for a year was noted in 1937. As the plants were immature when sampled, the results, theoretically, should have been higher. The results for 1937 indicate a condition lacking sufficient nitrogen for normal growth and development.

Surveys of these plots have shown quite clearly that a deficiency of nitrogen is apparent during the very early stages of growth and that this condition is independent of nitrogen additions. This would indicate a profound degree of competition between the organisms affecting the decay of the more or less resistant old legume residues and the growing tobacco for this particular element. This nitrogen, utilized by the microörganisms and built into their cells, is eventually released for plant use, but this may or may not be beneficial to the crop, depending upon its physiological age at the time of release.

EFFECT OF FERTILIZER PHOSPHORUS ON THE YIELD AND PHOSPHORUS CONTENT OF TOBACCO AS INFLUENCED BY THE ROTATION

Plots 3, 8, and 9 received varying quantities of phosphoric acid, the concentration of both nitrogen and potassium being held constant in these mixtures. The results obtained on yield of these plots are presented in table 9.

While some degree of inconsistency of results is evident, it is apparent that augmenting the phosphoric acid content of the mixture applied has a beneficial effect on growth. These and other studies lead to the belief that at least 80 pounds of actual phosphoric acid per acre are necessary for proper growth and development.

Table 9.—Influence of phosphoric acid additions on yields of tobacco; Roseville, 1935-40.

Plot No.	Fertilizer Treatment		Average Yield 1935 to 1940	Rank of Yield 1935 to 1940		
	Quantity	Formula		Lowest	Highest	Average
	pounds per acre		*pounds per acre*			
8	1,000	6-0-12	1,538	29th	14th	23rd
9	1,000	6-4-12	1,546	25th	6th	20th
3	1,000	6-8-12	1,757	17th	1st	5th

Chemical analyses of many samples of mature tobacco leaves, including those of the 3-year rotation plots, indicate an average phosphorus content of 0.25 per cent. Rarely was a sample found containing more than 0.30 per cent or less than 0.20 per cent.

EFFECT OF FERTILIZER POTASH ON YIELD AND POTASSIUM CONTENT OF TOBACCO AS INFLUENCED BY ROTATION

Plots 1, 2, 3, and 4 received varying quantities of potash, while the concentrations of both nitrogen and phosphorus were held constant. The effect of these treatments are given in table 10.

Table 10.—Influence of potash additions on yield of tobacco; Roseville, 1935-40.

Plot No.	Fertilizer Treatment		Average Yield 1935 to 1940	Rank of Yield 1935 to 1940		
	Quantity	Formula		Lowest	Highest	Average
	pounds per acre		*pounds per acre*			
1	1,000	6-8-4	1,699	15th	4th	10th
2	1,000	6-8-8	1,755	20th	3rd	6th
3	1,000	6-8-12	1,757	17th	1st	5th
4	1,000	6-8-16	1,696	16th	7th	12th

Without manure, the increased quantity of potash applied did not materially affect the yield. As a matter of fact the yield obtained with 40 pounds per acre of potash was approximately the same as when 160 pounds were applied. Better results were obtained on plot 26, where manure was used with the same quantity of fertilizer potash.

A study of the potassium content of the mature tobacco over a 5-year period was made, the results of which appear in table 11. The figures presented indicate quite clearly that under the conditions of these experiments, the quantity of potassium supplied the soil is by no means reflected by an increased quantity in the leaves. It may be noted that the potassium content averaged less than 3 per cent over this period, far less than the quantity desired for yield, quality, and resistance to leafspot disorders.

The samples of immature tobacco obtained in 1937 averaged somewhat higher than those taken in the other four seasons. Here again, however, are found marked inconsistencies. For example, plot 19, a no-treatment plot, produced tobacco containing a higher

Table 11.—Potassium content of mature tobacco (sixth leaf) on a moisture-free basis, grown on the 3-year rotation plots over a period of five years; Roseville, 1935-39.

PLOT NO.	1935	1936	1937	1938	1939	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.10	1.73	2.47	2.31	1.39	2.00
2	2.59	2.55	3.21	2.53	1.59	2.49
3	2.46	3.36	3.49	2.78	2.11	2.84
4	3.16	3.74	4.01	2.80	2.74	3.29
5	2.61	3.88	3.91	2.20	2.42	3.00
6	2.30	3.16	3.99	3.35	2.06	2.96
7	2.85	2.56	2.99	2.78	2.15	2.67
8	2.28	3.10	3.83	2.42	2.32	2.79
9	2.62	2.60	4.36	2.47	2.01	2.81
10	2.36	3.39	4.10	2.36	2.33	2.91
11	1.98	2.42	2.72	1.80	2.03	2.19
12	3.05	3.62	3.85	2.58	2.77	3.17
13	2.63	3.10	3.24	2.62	2.32	2.78
14	2.41	3.02	3.18	1.96	2.01	2.52
15	1.91	2.81	3.94	1.83	1.86	2.47
16	1.57	2.21	2.01	2.21	1.92	1.98
17	2.47	2.91	2.49	3.10	2.37	2.67
18	3.04	3.30	3.30	3.69	3.57	3.38
19	1.02	2.37	2.56	0.94	0.97	1.57
20	2.29	2.91	3.28	2.22	1.99	2.54
21	3.54	3.53	4.50	3.47	2.45	3.50
22	4.02	3.98	4.03	3.58	2.82	3.69
23	3.75	3.43	4.20	3.49	2.35	3.44
24	4.31	3.78	4.30	3.56	2.57	3.70
25	2.66	3.40	3.27	2.32	2.06	2.74
26	4.11	4.02	3.78	4.19	2.91	3.80
27	3.79	2.98	3.30	3.94	1.90	3.18
28	4.41	3.96	4.02	4.50	2.51	3.88
29	1.91	2.04	2.05	1.84	1.11	1.79
30	3.46	2.37	3.40	2.64	2.29	2.83
31	3.11	2.63	4.18	3.51	2.80	3.25
AVERAGE	2.80	3.06	3.49	2.77	2.22	2.87

concentration of potassium than was found in tobacco from plot 1 receiving 1,000 pounds per acre of a 6-8-4 mixture applied broadcast, plot 16 receiving 500 pounds per acre of a 6-8-12 mixture, and plot 17 receiving 1,000 pounds per acre of the same mixture applied to the row. It may be noted that low-nitrogen and low-phosphorus additions aided in the intake of potassium for that particular season.

Experience has shown that the intake of potassium by cigar-leaf tobacco in Lancaster county is governed largely by the quantity of available soil moisture present. Relatively small quantities of this particular element are absorbed during a drought, regardless of the quantity at the disposal of tobacco plants.

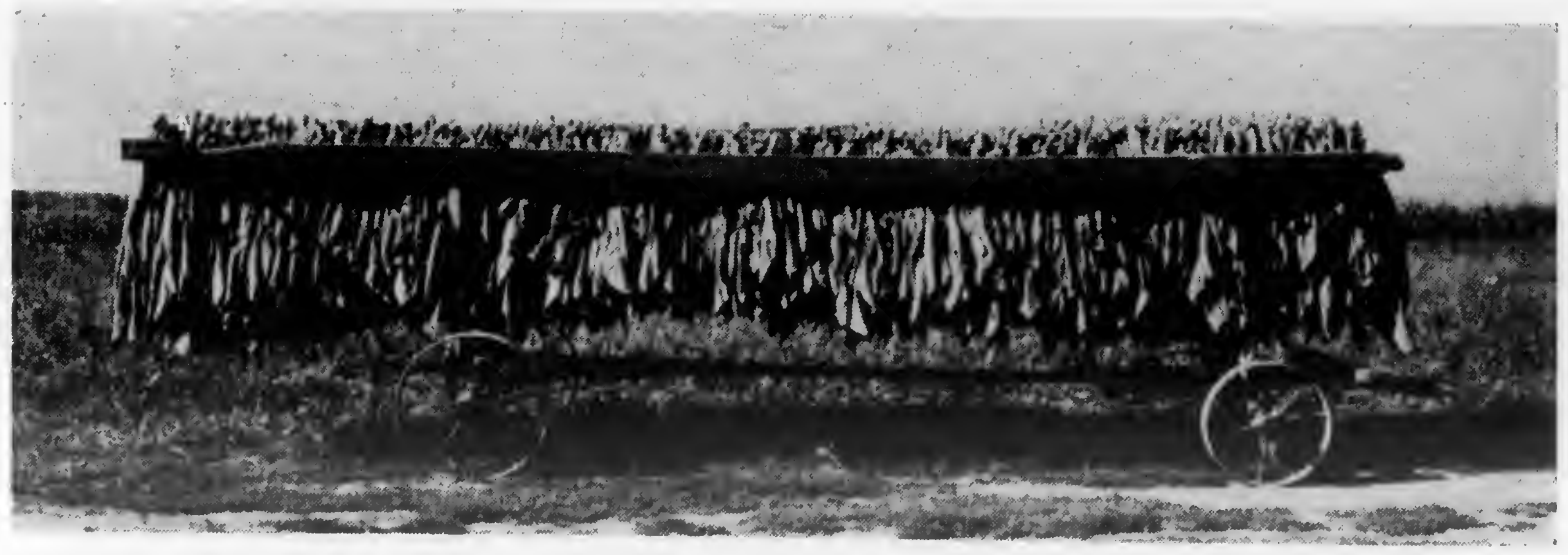


Fig. 3.—A load of Pennsylvania cigar-leaf tobacco, freshly harvested, as it comes from the experimental plots.

On the other hand, relatively large quantities of potassium have been found present in tobacco leaves where rainfall was both ample and well distributed and where the conditions for growth and development were otherwise satisfactory. However, the erratic results obtained regarding the absorption of both nitrogen and potassium must indicate the overshadowing effect of the preceding crop.

NITROGEN AND POTASSIUM CONTENT AND TOTAL YIELD OF CIGAR-LEAF TOBACCO AS INFLUENCED BY FERTILIZER TREATMENT WITH CORN AS THE PRECEDING CROP

Long before the 3-year rotation experiment was completed, it became apparent that legumes should not immediately precede tobacco in a rotation. Therefore, in 1938 a series of experiments was initiated in which corn preceded tobacco rather than legumes. This experiment was conducted over a 3-year period. Several of the treatments used were analogous to those used in the 3-year rotation experiment and may be used in comparison of the systems.

The soil chosen for these particular experiments was not in a state of high fertility and, in addition, was subject to considerable washing. On the other hand, the plots of the 3-year rotation experiment should have been in high fertility where the quantity of organic additions and fertilizers applied is taken into consideration. Nevertheless, the evidence submitted in table 12 tends to favor corn rather than legumes as the preceding crop for tobacco.

From table 12 it may be noted that plots receiving no commercial fertilizer gave a somewhat higher yield where legumes rather than corn constituted the preceding crop. However, in this case a higher concentration of both nitrogen and potassium were found in the tobacco plants where corn was the preceding crop. A greater nitrogen and a lesser potassium content were obtained from plots receiving 10 tons of manure alone following corn, the yield being

Table 12.—Effect of preceding crop on average yield of tobacco for 1938, 1939, and 1940, and the average nitrogen and potassium content of mature leaf for 1939 and 1940; fertilizer applied at the rate of 1000 pounds per acre where used; Roseville.

PRECEDING CROP	FERTILIZER FORMULA	MANURE	NO. OF PLOTS TESTED	AVERAGE YIELD	AVERAGE COMPOSITION FOR 1939 AND 1940	
					Nitrogen	Potassium
		tons per acre		*pounds per acre*	*per cent*	*per cent*
Legumes	6-8-12		5	1,530	3.32	2.56
Corn	6-8-12		4	1,628	3.51	2.52
Legumes	3-8-12		1	1,365	3.11	2.71
Corn	3-8-12		4	1,576	3.50	2.95
Legumes	6-0-12		1	1,372	3.52	2.37
Corn	6-0-12		2	1,428	3.96	2.90
Legumes	6-4-12		1	1,454	3.66	2.24
Corn	6-4-12		2	1,591	3.75	2.45
Legumes	None		2	1,315	3.70	1.22
Corn	None		2	1,249	3.85	1.47
Legumes	0-8-12	10	1	1,471	3.17	2.96
Corn	0-8-12	10	4	1,643	3.43	3.04
Legumes	3-8-12	10	1	1,558	3.26	3.20
Corn	3-8-12	10	4	1,743	3.65	3.29
Legumes	3-8- 8	10	1	1,477	3.38	2.92
Corn	3-8- 8	10	2	1,661	3.04	2.58
Legumes	3-8-16	10	1	1,726	3.27	3.55
Corn	3-8-16	10	2	1,759	3.26	3.85
Legumes	——	10	1	1,555	3.30	2.92
Corn	——	10	2	1,535	3.77	2.36
Legumes	——	20	1	1,710	3.30	3.51
Corn	——	20	2	1,664	3.65	2.45

practically the same. When 20 tons of manure alone were applied, a somewhat higher yield, a markedly higher potassium content, but a lower nitrogen content were obtained where legumes preceded tobacco. However, where different fertilizer mixtures were used alone or with manure, the results were decidedly in favor of corn as the preceding crop.

BURN AND QUALITY OF CIGAR-LEAF TOBACCO AS INFLUENCED BY FERTILIZER TREATMENT AND PRECEDING CROP

Samples of well-cured tobacco from the 1938 and 1939 crops on the 3-year rotation plots were tested by competent experts for burn and quality. The results of these tests are presented in table 13.

On the whole, the tests on burn and quality showed pronounced inconsistencies as may be noted in the case of plots receiving 1000 pounds per acre of a 0-8-12 mixture. Some of these, however, were much superior to those receiving the row treatments with well balanced mixtures.

Table 13.—Burn and quality of cigar-leaf tobacco as related to fertilizer treatment and legumes as the preceding crop; Roseville, 1938 and 1939.

PLOT NO.	BURN		QUALITY	
	1938	1939	1938	1939
1	Fair	Poor	Fair	Poor
2	Fair	Fair	Fair	Poor
3	Fair	Irregular	Fair	Very poor
4	Poor	Fair	Poor	Fair
5	Fair	Fair	Poor	Poor
6	Poor	Fair to poor	Poor	Poor
7	Fair	Good to fair	Fair	Fair to poor
8	Good	Fair	Fair	Poor
9	Poor	Fair	Poor	Fair to poor
10	Good	Fair	Fair	Fair
11	Fair	Very poor	Fair	Very poor
12	Fair	Irregular	Fair	Fair to poor
13	Fair	Fair to poor	Poor	Fair
14	Fair	Fair to poor	Fair	Poor
15	Good	Fair to poor	Fair	Poor
16	Poor	Poor	Poor	Poor
17	Poor	Poor	Poor	Poor
18	Poor	Good to fair	Poor	Fair to poor
19	Poor	Very poor	Poor	Very poor
20	Poor	Very poor	Poor	Very poor
21	Good	Fair to poor	Fair	Fair to poor
22	Good	Fair	Fair	Fair to poor
23	Fair	Fair to poor	Fair	Fair
24	Good	Good	Fair	Good to fair
25	Good	Good to fair	Good	Fair
26	Good	Good	Good	Fair
27	Fair	Good to fair	Fair	Fair
28	Good	Good to fair	Good	Fair
29	Poor	Very poor	Poor	Very poor
30	Fair	Fair	Fair	Fair
31	Fair	Good to fair	Fair	Fair

A study was made on tobacco where corn was the preceding crop in 1938 and 1939, and on the original 10-acre experimental field. These were the so-called "Potassium Placement Plots." The fertilizers applied to these plots contained the same nitrogen and phosphoric acid content, but the potash application was allowed to vary to a considerable degree. In addition, various methods of applying these mixtures were studied.

No manure was applied to the placement plots in 1938 but spent mushroom manure was applied in 1939 at the rate of 10 tons per acre. Neither of these treatments are to be recommended.

The results of these tests of burn and quality, accompanied by data on the nitrogen and potassium content of the mature tobacco, are presented in table 14.

Table 14.—Yield, and nitrogen and potassium content, of tobacco following corn as modified by field treatment and placement of fertilizers; basic treatment, 4-8-12 at the rate of 1,000 pounds per acre.

PLOT No.	FERTILIZER TREATMENT Formula	FERTILIZER TREATMENT Method of Application*	YIELD IN POUNDS PER ACRE 1938	YIELD IN POUNDS PER ACRE 1939	BURN 1938	BURN 1939	QUALITY 1938	QUALITY 1939	NITROGEN IN LEAVES 1938	NITROGEN IN LEAVES 1939	POTASSIUM IN LEAVES 1938	POTASSIUM IN LEAVES 1939
									per cent	*per cent*	*per cent*	*per cent*
1-C	4-8-35	Bands	1205	1696	Good	Good	Fair	Fair	1.99	4.29	3.25	3.12
2-C	4-8-35	Broken bands	1232	1891	Fair	Fair	Fair	Fair	2.11	4.19	3.60	3.03
3-C	4-8-12	Bands	1489	1762	Good	Fair	Fair	Fair	2.04	4.22	3.22	3.10
4-C	4-8-12	Broken bands	1397	1659	Good	Very good	Fair	Fair	2.46	4.02	3.59	2.85
5-C	4-8-12	Broadcast	1363	1473	Very good	Good	Fair	Fair	2.05	4.13	3.66	2.77
6-C	4-8-12	Drilled in row	1368	1630	Very good	Good	Good	Good	2.31	4.17	3.67	3.00
7-C	4-8-12	Drilled in row	1506	1656	Fair	Very good	Fair	Fair	2.20	4.45	3.89	2.49
8-C	4-8-35	Bands	1403	1776	Good	Very good	Fair	Good	1.91	4.36	3.56	3.57
9-C	4-8-35	Broken bands	1414	1866	Good	Fair	Good	Poor	1.85	4.53	3.96	3.69
10-C	4-8-12	Bands	1386	1720	Good	Good	Fair	Poor	2.21	4.48	3.41	2.97
11-C	4-8-12	Broken bands	1410	1707	Good	Good	Fair	Fair	2.23	4.54	3.68	3.09
12-C	4-8-12	Broadcast	1293	1525	Good	Good	Good	Fair	2.26	4.49	3.76	3.06
13-C	4-8-12	Drilled in row	1320	1508	Good	Good	Fair	Fair	2.30	4.49	4.41	3.09
14-C	4-8-12†	Drilled in row	1102	1297	Good	Good	Fair	Good	2.07	....	4.27	....

* Bands = continuous bands 3 inches from row.
Broken bands = 18 inches along each plant on both sides of row, 3 inches away.
Broadcast = Uniformly distributed and harrowed into the soil.
Drilled in row = Placed in rows beneath the plants.

NOTE: When 35 units of potash were used the first application of 12 units were followed by 12 and 11 additional units during the growing period.

† 1500 pounds per acre basic mixture. Low yields of plot 14-C due to border effect.

While it would be rather difficult to compare tobacco grown on the placement plots with those of the 3-year rotation experiment, it would appear that less nitrogen was absorbed by plants following corn in 1938 than tobacco produced on the 3-year rotation plots. However, in 1939 this condition was completely reversed. While the conditions appeared better for the absorption of potassium from the crops following corn, the concentration found in the leaves could not be correlated with the amount supplied in the fertilizer. However, the burn and quality of the tobacco following corn were much superior and more consistent than the tobacco following legumes.

DISCUSSION

The demand upon the soil by cigar filler tobacco for moisture and mineral nutrients is very great. Yields of cigar filler tobacco must be much larger than yields of certain other types if the grower is to receive a profit from the crop because of the value of a pound of cigar-filler tobacco is usually much less than that of many other important types of tobacco. The economic necessity of high yields is not the only factor which tends to make the demand of cigar-filler tobacco upon the soil inordinately great. Good quality in tobacco of certain other types, as for example flue-cured tobacco, is associated with a nitrogen content of only 2 per cent and a potassium content comparable in amount. Excellence in quality in cigar-filler tobacco, on the other hand, is associated with a nitrogen content of from 3.5 per cent to 4.25 per cent and a potassium content at least as great.

Consideration of both yield and chemical composition reveals that the moisture demands of the cigar-filler type are at least double the demands of the lower nitrogen tobaccos and the nitrogen and potassium demands upon the soil may be as much as from two to four times as great. As a result of these great demands upon the soil for moisture and nutrients, the culture of cigar-filler tobacco is conducted upon fertile, heavy soils such as are found in the Lancaster area. The maintenance of the necessary level of fertility in these soils requires frequent application of nutrients, and the maintenance of tilth and moisture-holding capacity requires frequent utilization of either plant or animal manures or both.

As an aid in maintaining a high level of available nutrients in the soil, particularly nitrogen, and in maintaining good tilth and moisture-holding capacity through the more frequent incorporation of organic matter, the 3-year rotation in which tobacco follows a legume was introduced experimentally. The results obtained over a period of nine years have been presented in this bulletin, and these have been compared with data obtained from plots following corn which received comparable treatments. This comparison shows that the attempt to produce tobacco upon soil in which a legume has immediately preceded the crop has proved disappointing.

In the case of most of the fertilizer treatments, as shown in table 12, the yield following corn exceeded that following a legume. Only in the case of inadvisable practices such as are represented by "No treatment" and "Manure alone" plots did the yield following a legume prove larger than that obtained on corn land, and in these cases the difference proved slight. The presence of the legume stubble in the soil had a depressing effect upon crop yield.

The tobacco grown on the plots of the 3-year rotation showed a certain lack in quality throughout the course of the experiment. This deficiency became somewhat more pronounced through the years. This lack of quality is shown in table 13. This confirms the belief of many Lancaster County growers who as the result of past experience avoid alfalfa stubble land in the selection of sites for tobacco.

Success in the production of tobacco in the Lancaster area is dependent not only on obtaining high yield and good quality but in addition upon the avoidance of serious leafspot damage in those years of erratic precipitation. From this standpoint the 3-year rotation also proved extremely disappointing. In every season in which leafspot damage assumed economic importance, the tobacco on the 3-year rotation plots suffered more severely than the tobacco which followed corn.

The chemical analyses performed in the course of this investigation are of particular interest, tables 8, 11, and 12. Among the reasons for the introduction of the 3-year rotation was the need of increasing the nitrogen content of the crop in order to reach the desirable range of from 3.5 to 4.25 per cent nitrogen. The introduction of the legume immediately preceding the tobacco crop, however, not only failed to result in increased nitrogen assimilation by the tobacco but actually resulted in the production of lower nitrogen tobacco. As shown in table 12 tobacco produced on corn land in most instances had a higher and more desirable nitrogen content than did tobacco produced under the 3-year rotation. Likewise potassium assimilation was depressed on those plots in which leguminous material had been turned under immediately preceding the planting of tobacco.

SUMMARY

Studies were conducted over a period of nine years as to the influence of fertilizer treatment on the yield and quality of Pennsylvania cigar-leaf tobacco and as modified by the preceding crop. As a result of these investigations the following conclusions seem justified:

1. The influence of a legume as the preceding crop overshadowed the effect of a given fertilizer treatment. The presence of legume stubble had a depressing effect upon both yield and quality.
2. Regardless of the fertilizer treatment, both the nitrogen and potassium content of the mature plants were usually too low for satisfactory yield and quality. This held true regardless of the fact that in most instances relatively large quantities of both nitrogen and potassium were supplied the plants.
3. The influence of corn as the preceding crop was definitely superior to that of legumes from the standpoint of yield, quality, and absorption of nitrogen and potassium.
4. The effect of legumes as the preceding crop were modified to a considerable extent by the amount and distribution of rainfall as indicated by seasonal difference in yield and composition.
5. Cigar-leaf tobacco requires a considerable amount of moisture for its normal development, but the rainfall should be well distributed throughout the entire growing season. The abrupt breaking of a drought usually results in tissue breakdown. This condition, however, is dependent on the physiological age of the plant; young plants being less susceptible than are those approaching maturity. In every season in which leafspot damage assumed economic importance, the tobacco following legumes suffered more severely than tobacco following corn.

BIBLIOGRAPHY

(1) Olson, Otto 1933. A report on tobacco investigations for 1933. *On file. The Pennsylvania State College.*

(2) Olson, Otto 1937. A report on tobacco investigations for 1937. *On file. The Pennsylvania State College.*

(3) Thomas, J. J., D. E. Haley, and Otto Olson 1936. The absorption of plant nutrients in relation to the production of Pennsylvania cigar-leaf tobacco. *The Pennsylvania Agricultural Experiment Station Bulletin* **331**.

Wartime Problems of Feeding and Nutrition

R. C. MILLER, Ph.D.
State College, Pennsylvania

Reprint from Journal of the American Veterinary Medical Association,
Vol. CII, No. 793, April, 1943, pp. 300-303.

Wartime Problems of Feeding and Nutrition

R. C. MILLER, Ph.D.

State College, Pennsylvania

A PRACTICAL discussion of nutrition and livestock feeding, as well as feeding itself, is difficult at the present time owing to the general unavailability of the many feedstuffs which have been considered essential for a sound feeding program.

Nevertheless, in spite of difficulties, the livestock industry is called upon to increase production of livestock products in 1943 to insure the continuance of the task undertaken by the nation.

There are shortages, per animal unit, of many feeds and supplements. There is shortage of farm labor. There are bottlenecks and priorities, ceilings in prices and restrictions on transportation, rumors and actualities. The outlook is uncertain but there is a bright side also. Veterinary science and biochemistry have made notable advances in recent years. Today, the animal industry is better equipped than ever before with knowledge and with some of the tools required to deal with the problem.

Finally, there is no restriction on the plain common sense which is going to play a big rôle in the solution of this problem. With the use of common sense combined with science, there are three things which will yield large returns to livestock production and not require the expenditure of much money or of the restricted materials:

1) The first is to stress, as never before, the paramount importance of the best sanitary conditions for all classes of livestock. Interest in sanitation should focus upon control of infectious and parasitic diseases. It is wasteful to try to properly nourish animals that are parasitized and sanitation is the chief step in the prevention of that condition.

2) Next, the greatest possible use of pasture should be emphasized — that is, good pasture, not only an exercise lot, although that is worth something. It is known that good permanent pasture can not be developed in one year or for one season but there are forage crops such as perennial rye grass that will serve as emergency pasture. Alfalfa is preferred but other legumes such as sweet clover and ladino clover (which is comparatively new in some sections) give good returns.

There is a shortage of fertilizer and fencing is scarce, but in any event, the use of all the pasture possible for all classes of livestock is helpful. Green grass and forage contain something which has not been fully identified or duplicated. Pasture furnishes something that can not be bought in a bag. Its greatest use will bring good returns in terms of growth, vigor, and health. To prolong the use of green grasses throughout the year, grass silage, which can now be made without the use of molasses or acid, will bring good returns in a limited feeding program.

The fullest use of pasture embraces another example of common sense which doesn't cost much and is too often forgotten, and that is plenty of sunshine. Many farmers are very stingy with sunshine for their livestock. As pointed out below, the vitamin D obtained through sunshine reduces the amounts of some of the minerals required and allows the limited quantities which can be provided by the feed, to be better utilized. There was never a time when year-around sunshine for livestock would bring bigger dividends.

At this point, it should be emphasized that increased production quotas will not be attained unless every livestock producer responds. Most of the large producers are already doing everything possible and, probably, increases can not be expected from them. The small producer must now be reached—those with 2 to 5 cows and a few pigs or chickens. Many of this group can redouble their efforts. The responsibility of increasing production falls to them.

3) Foremost among the specific problems confronting feeders is that of protein. This is especially serious from the viewpoint of total protein supply and sources. Conditions imposed along our coastlines by warfare have greatly decreased the production of fish meal, and importations of animal protein have been restricted. Domestic tankage production is good but the total supply per animal is below the present needs. Fortunately, the animal-protein problem does not apply to all livestock. The microörganisms in the digestive tract of ruminants supply these animals many essential amino acids, or protein fragments, required for growth and development. This is not true in animals with a simpler digestive tract. In swine and poultry, the animal-protein problem is of great concern. Whereas, cattle and sheep thrive on plant protein, pigs and chickens have requirements for animal proteins which, unfortunately, war does not change.

The best substitute for tankage, meat scraps, and fish meal as far as protein is concerned appears to be properly processed soybean oilmeal. The quality of protein it supplies seems to be adequate for swine and poultry. The protein content of their rations can be maintained by its use.

It is not known how far the supply of soybean oilmeal will extend in the present feeding program. The demand may exceed the supply, but effort is being made to increase the supply as much as possible. Soybeans are being shipped to the South to take advantage of the southern mills which, ordinarily, are used only a part of the year for crushing and pressing peanuts and cottonseed.

The animal protein available should be conserved by including it only in the rations of young, growing animals. Once they are off to a good start with growth well under way, the shift should then be made to plant protein as it will suffice for finishing and fattening meat animals.

Although the dairyman does not need animal protein, he must use his ingenuity to keep up, as well as he can, the protein content of his feeds by the wise use of soybeans, linseed, and gluten. The use of roughages such as alfalfa will help out.

What are the dangers of changing from animal to plant protein in feeding swine? (The question may also be asked of poultry.) First, vegetable proteins are deficient in the calcium required for bone development. Ordinarily, bone meal would be used in this situation. But, the present supply of bone meal is inadequate and, therefore, can not be depended upon. Limestone is the next choice as a source of calcium but it does not supply phosphorus. Therefore, its use in unlimited amounts is out of the question. The use of limited amounts of limestone (1 to 1.5% of the ration) is, however, practicable. The mineral situation will be partly taken care of in this way, especially if the animals receive adequate vitamin D.

For swine and cattle as mentioned above, sunshine will serve the purpose of supplying vitamin D to good advantage. Irradiated yeast, a relatively low-priced product, may also be used for four-footed animals. Fish oils, if available, will serve the purpose for poultry. If not available, a synthetic vitamin D of the new activated, animal-sterol type is satsifactory. Another possible substitute for bone meal is rock phosphate. This natural product supplies

---

Presented at the fifty-ninth annual meeting of the Veterinary Medical Association of New Jersey, Trenton, Jan. 14, 1943.

From the Department of Biochemistry, Pennsylvania State College.

both calcium and phosphorus but caution must be used in the particular product fed, since much of the rock phosphate contains fluorine in toxic amounts. The manifestation of this toxicity are thickening, softening, and weakening of the bones, associated with characteristic histological changes. Defects occur also in the teeth which become soft and worn. The results are lowered food consumption, impaired growth, and diminished milk production as well as breeding difficulties in the case of cattle. These conditions do not develop rapidly but are inevitable after prolonged high intake of fluorine. There is available a limited amount of rock phosphate which has had the fluorine removed. This is perfectly safe to feed. Where rock phosphate is used as a mineral supplement, its fluorine content must always be considered. No mineral supplement containing more than 0.1 per cent of fluorine should be fed.

A deficiency of vitamin A may be found in a present day feed. White corn and white-capped corn rations are low in carotene, the precursor of vitamin A, unless provision is made to supply that factor. Yellow corn is the most universally and generally available source of carotene and should, therefore, be used if available. Again, as in the instance of animal protein, these products should be fed mostly to the young, growing animals. Fortunately, vitamin A can be stored in the organs and tissues of most animals. So, if a continuous supply of vitamin A is not available, the body reserves can carry the animals over for a period. Pasture enters this picture, particularly good, green forage which is an excellent source of vitamin A, or rather of its precursor, carotene.

In respect to vitamin B, restricted feeding conditions do not help the situation. In the case of cattle and other ruminants, these vitamins are not a problem owing to the synthesis of vitamin B factors in the digestive tract. There is evidence, however, that swine need, at least, five of the B vitamins: thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine. In the past, it has been supposed that these factors were supplied in the ordinary good ration. With a restricted ration, however, B-factor deficiencies may easily occur. These are manifested by unthriftiness, rough skins and coats of hair, scouring, vomiting, goose stepping and incoördination, and growth failure.

Dried brewers' yeast should supply the recognized B factors for swine, but liver meal is the only thing discovered thus far which supplies some of the unknown factors. It is sometimes thought that the ordinary, good ration will supply all of the B factors needed by growing pigs as long as the feed is consumed in sufficient amounts. Should anything like influenza or chilling cause a reduction of the feed intake, these deficiencies may become apparent. It appears that many of our good rations may be adequate though not rich in the amount of B factors they furnish. Sometimes the situation can be saved by the use of a single B vitamin, niacin for instance. Anything that restores appetites and brings feed consumption up to normal will correct matters. There has been some success in fermenting the feed of swine—going back to the old swill-barrel idea which may not be at all bad.

In poultry, the demands for B vitamins are, perhaps, more exacting than in swine. Apparently, factors in addition to those enumerated for swine are required. It is necessary to select carefully the ingredients of poultry rations and to use vitamin B carriers such as skimmed milk powder, distillery by-products, and yeast to satisfy these requirements.

The distillery by-products are a good source of some of the B factors for both swine and poultry. They are residues of alcohol production—dried distillers' solubles, and dried distillers' grains (30% protein) with the solubles added. They are suitable for swine and furnish some of the B factors required by poultry. These products are relatively new sources of vitamins and while not available in unlimited quantities, they will apparently serve a partial source of supply of some of the B factors from now on. In addition, some of the pure synthetic B vitamins are now becoming cheap enough to warrant consideration of their regular use in some rations.

With further respect to B vitamins, there may be danger in the present plan involving the use (in the livestock feeding program) of large amounts of the surplus wheat which has been in storage for some time. It is probable that this wheat has lost some of its nutritive value during storage, so that it is not, as is often claimed, equal to corn as a replacement for a part of the corn in the ration. Wheat lacks the vitamin A contained in yellow corn. Storing possibly lowers some of its content of vitamin B factors. Such wheat should be used cautiously and never substituted for more than one-half of the corn in a ration. A gradual substitution, beginning with a small amount, is wise in any case.

Last summer, I heard a definition of a vitamin which expresses an interesting point of view:

> A vitamin is an alleged component of a food [feed], drug, or cosmetic which is placed in the product by the advertising department, and assayed in terms of sales resistance. An underdosage leads to an increased sale of the competitor's product, and an over-dosage leaves a bad taste in the mouth of the consumer.

This definition is given because in the present situation with respect to the feeding of livestock, it is felt that the industry [livestock] may be confronted with the exploitation of all sorts of supplementary products whose real value may be difficult to appraise. It is a difficult and complex situation which will not be solved by any one thing that comes in a bag or bottle. Rather, the best judgment and coöperation of all who are qualified will be needed to carry through a livestock feeding program which will even begin to fulfill our needs, not only during 1943, but also during the next few years.

Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to *Tetranychus telarius* (L.)[1,2]

H. L. King and D. E. H. Frear, *Pennsylvania Agricultural Experiment Station, State College*[3]

In the search for new organic insecticides, the highly toxic properties of nicotine have stimulated a number of investigations of the insectidal possibilities of other N-heterocyclic compounds. Several workers have studied the properties of pyrrole and its derivatives, some have studied the various dipyridyls, and others have investigated the derivatives of pyridine and quinoline. The last-mentioned group of compounds has been tested by a number of different methods and on a number of different insects. Richardson & Smith (1923), in an investigation of a large number of organic compounds, sprayed the materials on nasturtium plants infested with bean aphids and considered their results as an indication of contact toxicity. Tattersfield & Gimingham (1927) sprayed aphids with certain of these compounds and confined the aphids in closed containers with leaves of their food plants. They considered their results from the standpoint of combined contact and fumigant action. Bushland (1940) mixed the test compounds with the culture medium in which he reared larvae of the screwworm, *Cochliomyia americana* C. & P., and in this manner measured the combined contact action and stomach action of the materials. Shaw & Steer (1938) investigated the ovicidal properties of certain quinoline derivatives, using eggs of *Orgyia antiqua* L., while Smith (1940) studied the toxicity of a group of related compounds to eggs of *Lygaeus kalmii* Stål.

The purpose of the investigations reported in this paper[4] was to measure the relative toxicity of pyridine and a series of compounds related to pyridine as contact sprays on the greenhouse red spider, *Tetranychus telarius* (L.); to attempt to establish some relationships between chemical structure and toxicity to red spider; and to compare these relationships with those established by the above investigators in their work with various insects.

Materials.—The choice of the greenhouse red spider, or spider mite, as a test animal was prompted by several considerations. It is an easily reared organisn, of economic importance, and is related to insects, but since it belongs to another class, it cannot be assumed that it would react to toxicants in the same manner as the insects which have been investigated by the workers mentioned above. Furthermore, it has sucking mouth-parts, thus eliminating to a great extent the possibility of stomach poisoning, and it is not particularly susceptible to fumigants. In this work the sprayed plants were kept in a well ventilated position, minimizing the possibility of fumigant action, so that the results represent contact action only.

The compounds tested in this work are listed in table 1. The chemicals used were all pure with the exception of β-picoline, which was an 85 per cent solution. Acridine and 2-amino-pyridine were crystalline preparations. All others were liquids. For testing, the compounds were dissolved in a mixture of equal parts of acetone and water. Each compound was in true solution at the time of spraying.

Table 1.—Degree of control of red spider secured by a 1.0% solution of various N-heterocyclic compounds.

Compound	Boiling Point (Degrees C.)	Per Cent Control of Red Spider
Pyridine	115.3	0.6
α-picoline	128	0.6
β-picoline	143.5	0.0
2-6-lutidine	143	1.3
2-me-6-amino-pyridine	208–9	39.6
2-amino-pyridine	204	41.2
Quinoline	237.7	65.1
Acridine	346	100.0

The sprays were applied from an atomizer-type air-brush, operated by a *De-Vilbiss* air compressor, at a constant spray-

---

[1] Authorized for publication on November 9, 1942 as Paper No. 1140 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Eastern Branch Program, New York, November 1942.

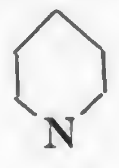
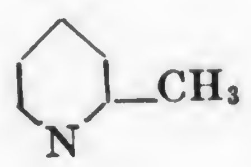
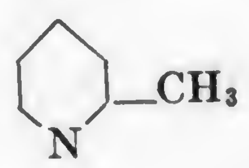
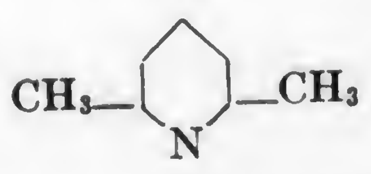
[3] Reilly Research Fellow and Assistant Professor of Agricultural and Biological Chemistry, respectively. This work was supported in part by a grant from the Reilly Tar and Chemical Corporation, of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry in cooperation with the Department of Zoology and Entomology, and at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

[4] Other papers are to follow.

ing pressure of sixteen pounds per square inch, and were sprayed into a wooden chamber, 30×39×35 inches, equipped with an exhaust fan at the back and a turntable at the rear center.

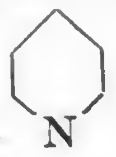
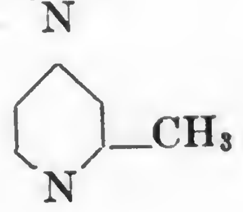
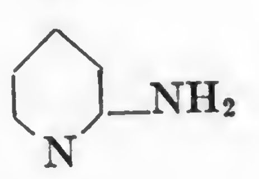
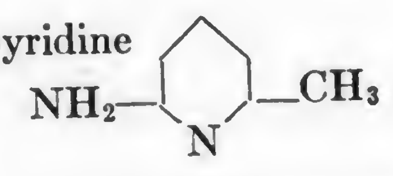
PROCEDURE.—Garden beans of the Black Wax Pencil Pod variety were grown in greenhouse flats until the seed-leaves were well developed, and were then transplanted into 2-inch pots. One day after transplanting, the plants were innoculated with mites by laying heavily infested leaves from the culture-bed on the plants to be innoculated. Twenty-four hours after innoculation, an infested plant was placed on the turntable of the spray-chamber and the spray applied from a distance of 30 inches, for 30 seconds. During this time the turntable and plant made approximately eight revolutions. After spraying, the plant was set aside for 24 hours, at the end of which time the numbers of live and dead adult mites were counted, using a 7-power binocular microscope. Per cent control was calculated by Abbott's formula, each day's results being corrected against a series of blank sprays containing equal parts of acetone and water. The survival of mites on the plants sprayed with the blank sprays was consistently above 90 per cent. Throughout the experiment, the average infestation per plant was well over a hundred mites.

Table 2.—Effect of the addition of methyl groups to the pyridine nucleus on the degree of control of red spider.

COMPOUND	STRUCTURE	PER CENT CONTROL OF RED SPIDER BY A 5 PER CENT SPRAY
Pyridine		1.5
α-picoline		0.7
β-picoline		2.2
2-6-lutidine		1.6

EXPERIMENTAL DESIGN AND RESULTS.—As the experiment was originally set up, each compound was tested at a concentration of 1 per cent, using from four to seven replicates, depending on the degree of agreement among them. The results of this series of sprays are given in table 1. It will be seen from the table that, at this concentration, the control given by pyridine, the picolines and lutidine was too low to demonstrate differences among them. Accordingly, these compounds were retested at a concentration of 5 per cent, using seven replicates. (Table 2.)

Table 3.—Comparison of the effect of the addition of amino and methyl groups to the pyridine nucleus on the degree of control of red spider.

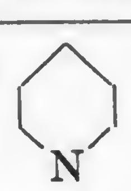
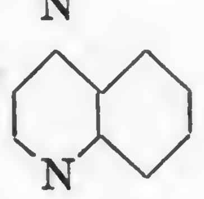
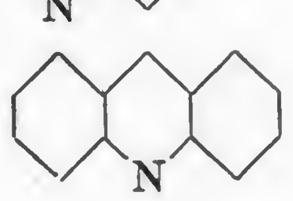
COMPOUND	STRUCTURE	PER CENT CONTROL OF RED SPIDER BY A 1 PER CENT SPRAY
Pyridine		0.6
α-picoline		0.6
2-amino-pyridine		41.2
2-me-6-amino-pyridine		39.6

DISCUSSION.—The compounds tested fall naturally into three series. The first of these is represented by table 2. Here is found an indication that the addition of one or two methyl groups does not increase the toxic action of the pyridine nucleus. However, it should be noted that even at a concentration of 5 per cent, these compounds did not give any significant degree of control. For this reason comparisons within the series may not be valid. Richardson & Smith (1923), experimenting with aphids, found picoline more toxic than pyridine, while Tattersfield & Gimingham (1927) found pyridine, picoline and lutidine to increase in toxicity in the order mentioned.

The second series of compounds is represented by table 3. Here it will be seen at once that the addition of the amino group to the α-position of the pyridine nucleus markedly enhances the acaricidal action

of that nucleus. The addition of a methyl group in the α-position, either to pyridine or to α′-amino-pyridine adds nothing to the toxicity of the original molecule. As mentioned above, a comparison of pyridine and picoline may be questioned because of the slight degree of control given by either. The comparison between 2-amino-pyridine and 2-me-6-amino-pyridine, however, is not subject to such criticism, and is taken as a definite indication that the methyl group does not add to the toxicity of the molecule.

Table 4.—Effect of the addition of benzene rings to the pyridine nucleus on the degree of control of red spider.

COMPOUND	STRUCTURE	PER CENT CONTROL OF RED SPIDER BY A 1 PER CENT SPRAY
Pyridine		0.6
Quinoline		65.1
Acridine		100.0

The third series of compounds is listed in table 4 and illustrates the remarkable increase in toxicity that occurs when one or two benzene rings are condensed with the pyridine nucleus. In the limited series studied, toxicity was almost directly proportional to the number of benzene rings added. In connection with this series, it may be mentioned that Smith (1940), as well as Richardson & Smith (1923), found quinoline more toxic than pyridine, while Tattersfield & Gimingham (1927) found pyridine, quinoline and acridine to increase in toxicity in the order mentioned.

The increasing order of toxicity of all the compounds studied is as follows: pyridine = α-picoline = β-picoline = 2-6-lutidine < 2-me-6-amino-pyridine = 2-amino-pyridine < quinoline < acridine. In so far as the same compounds were used, this order agrees in a general way with the order of toxicity given by Tattersfield & Gimingham and by Richardson & Smith. However, the authors mentioned, working with aphids, were able to demonstrate differences among the lower members of the series, indicating that the methyl groups added to the toxicity of the parent molecule. In the present investigation with greenhouse red spider, this was not the case, indications being found that the methyl group did not add to the toxicity of the molecule to this organism.

The boiling points of the compounds used are listed in table 1. It will be noted that the order of toxicity is approximately the same as the order of increasing boiling points.

SUMMARY.—Eight N-heterocyclic compounds related to pyridine were tested as contact acaricides on the common red spider, *Tetranychus telarius* (L.).

Results of these tests indicated that

(a) the addition of one or two methyl groups did not increase the toxicity of the pyridine nucleus.

(b) the addition of an amino group in the α-position markedly increased tbe toxicity of the molecule to the mite.

(c) the condensation of the pyridine nucleus with a benzene ring to form quinoline resulted in increased toxicity, while condensation with two benzene rings to form acridine further increased the toxic action toward the mites.

The increasing order of toxicity of all the compounds tested is as follows: pyridine = α-picoline = β-picoline = 2-6-lutidine < 2-me-6-amino-pyridine = 2-amino-pyridine < quinoline < acridine.

The increasing order of toxicity was found to be nearly the same as the increasing order of boiling points.—12-2-42.

LITERATURE CITED

Bushland, Raymond C. 1940. The toxicity of some organic compounds to young screwworms. JOUR. ECON. ENT. **33**(4): 669–76.

Richardson, C. H., and C. R. Smith. 1923. Studies on contact insecticides. U.S.D.A. Bul. **1160.**

Shaw, H., and H. Steer. 1938. Studies on the toxicity of certain nitrophenols, thiocyanates, naphthalene derivatives and organic bases to the eggs of some common orchard pests. Jour. Pomol. Hort. Sci. **16:** 364–88.

Smith, Cass F. 1940. Toxicity of some nitrogenous bases to eggs of *Lygaeus kalmii.* JOUR. ECON. ENT. **33**(5): 724–7.

Tattersfield, F., and C. T. Gimingham. 1927. Studies on contact insecticides. V. The toxicity of tl e amines and N-heterocyclic compounds to *Aphis rumicis* L. Ann. Appl. Biol. **14:** 217–39.

Reprinted August 2, 1943 from
JOURNAL OF ECONOMIC ENTOMOLOGY **36:** (2):263

Reprinted from THE JOURNAL OF NUTRITION
Vol. 26, No. 2, August, 1943

PREVENTION OF PEROSIS AND DERMATITIS IN TURKEY POULTS [1]

H. PATRICK, R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL
Departments of Agricultural and Biological Chemistry and Poultry Husbandry, The Pennsylvania State College, State College, Pennsylvania.

(Received for publication October 19, 1942)

It is recognized that manganese is necessary for the prevention of perosis in chicks (Wilgus, Norris and Heuser, '37); however, Jukes ('40) found both manganese and choline essential for protection of poults from perosis. Jukes ('40), Hogan et al. ('41) and Record and Bethke ('42) established the essential rôle of choline in the prevention of perosis in chicks.

It was recently reported that in addition to choline, there exists at least two other organic nutrients concerned in the prevention of perosis in chicks. One of these substances is biotin (Jukes and Bird, '42), and the other substance (Richardson and Hogan, '41), not identical with any of the recognized vitamins, is present in the aqueous extract of desiccated beef liver. This unrecognized substance was adsorbed on fuller's earth and subsequently eluted with ammonium hydroxide. Perosis has been reported to accompany the egg white injury syndrome in chicks (McElroy and Jukes, '40), which suggests that this symptom is a result of induced biotin deficiency.

Hegsted et al. ('40, '42) and Ansbacher and Landy ('41) established the essential rôle of biotin in the prevention of a scaly dermatitis in chicks which was distinct from the dermatitis produced by pantothenic acid deficiency. Lepkovsky and Jukes ('36) and Jukes ('38) reported that vitamin G was an

---

[1] Authorized for publication on October 1, 1942, as paper no. 1130 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This research was aided by a grant from the Cooperative G. L. F. Exchange, Inc., Ithaca, N. Y.

'anti-dermatitis' vitamin in poult nutrition. Patrick, Boucher, Dutcher and Knandel ('41) reported that poults receiving a simplified ration containing a yeast residue or crystalline biotin methyl ester did not develop dermatitis even when the ration was grossly inadequate in riboflavin. Supplementation with 8 µg. of biotin daily gave complete protection. Perosis was frequently observed in this laboratory in turkey poults which received rations adequate in choline and manganese. Dried brewers' yeast was found to be effective in preventing perosis under these conditions. This paper reports studies of the antiperosis properties of yeast fractions, crystalline biotin methyl ester and choline. Further evidence on the effectiveness of biotin and ineffectiveness of riboflavin in the prevention of dermatitis is also presented.

EXPERIMENTAL

The composition of the simplified basal ration is given in table 1. Bronze and White Holland turkey poults were used throughout the experiment. They were started when 1 day old and kept in electrically heated brooders equipped with

TABLE 1

Composition of the simplified basal ration.

	%
Purified casein	25
Extracted wheat bran	20
Salt mixture no. 30 [1]	4
Manganese sulfate	0.04
Soybean oil [2]	5
Cod liver oil (400 D)	2
Wheat germ oil [3]	2
Polished rice	5
Choline	0.2
Pyridoxine	0.0005
Thiamine	0.0005
Vitamin carriers as described in tables 2 and 3	
Corn starch to make 100%	

[1] Hubbell, Mendel and Wakeman ('37).

[2] Kindly furnished by Central Soya Co., Fort Wayne, Indiana.

[3] Kindly furnished by General Mills Inc., Minneapolis, Minnesota.

hardware cloth floors. The experimental ration and water were fed ad libitum.

The vitamin carriers were prepared from strain S dried brewers' yeast [2] as follows: (1) The yeast was extracted five times with 95% ethyl alcohol at 65°C. (2) The alcohol insoluble fraction was extracted ten times with boiling HCl-acidulated water at pH 4. (3) The residue was not treated further. (4) The acidulated aqueous extract from 2 was concentrated in vacuo and fed as such or fractionated in the following manner: (A) Alcohol-precipitate and filtrate-fraction: these fractions were prepared by adding enough 95% ethyl alcohol to obtain maximum precipitation and then separated by decantation. (B) Norite and fuller's earth eluates: these fractions were prepared by treating the acidulated aqueous extract, after being concentrated to a convenient volume, with norite or fuller's earth and eluting the adsorbates with 0.2% ammonium hydroxide, thus forming the norite or fuller's earth eluate.

The extracted wheat bran was prepared as follows: (1) Washed once with tap water at 60°C. (2) Washed five times with 0.1% ammonium hydroxide at 60°C. (3) Washed eight times at 60°C. with HCl-acidulated water at pH 4. (4) Washed with cold tap water until free of acid and then dried in a forced draft oven at 80°C.

Biotin concentrate I was prepared from liver residue according to the method of du Vigneaud et al. ('41), and biotin concentrate II (S.M.A.-200) and crystalline biotin methyl ester were purchased from a commercial concern.[3]

The influence of riboflavin on dermatitis and perosis

A summary of results obtained by feeding varying levels of riboflavin is presented in table 2.

Basal ration 1 had the following precentage composition: simplified basal 92 (table1), alcohol precipitate of yeast fraction 3, feeding cane molasses 5, and nicotinic acid 0.001. The

[2] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.

[3] S.M.A. Corporation.

alcohol precipitate of yeast fraction was added as a source of unrecognized vitamins in which the simplified basal ration was likely to be deficient. The feeding cane molasses was added as a source of pantothenic acid. The complete ration was assayed for riboflavin in two laboratories by the fluorometric method: the laboratory of the Cooperative G. L. F. Exchange, Inc., Buffalo, N. Y. reported 155 μg. per 100 gm.; 160 μg. per 100 gm. was found in this laboratory.

TABLE 2

Effect of graded levels of riboflavin on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 1 [1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMATITIS	PEROSIS
		%	%
Riboflavin			
None	16	87	25
200 μg.	12	92	50
230 μg.	17	76	53
260 μg.	16	75	56
290 μg.	18	72	33
320 μg.	18	67	28
350 μg.	15	67	67
380 μg.	11	45	36
380 μg. plus 10% dried brewers' yeast	15	none	none

[1] Riboflavin expressed as micrograms per 100 gm. of ration.

Severe dermatitis and perosis occurred frequently by the end of the third week in the poults of all groups receiving varying levels of riboflavin, while a control group of poults receiving 10% dried brewers' yeast developed normally. The dermatitis involved the feet, anus, mouth, eyes, and most proximal portion of the upper beak. The accumulation of exudate and the severity of the dermatitis around the eyes and mouth may have been aggravated by a secondary infection. In many instances, when the poults lived long enough, the surface of the skin appeared scaly and felt like parchment and the wings became rigid, due to dermatitis involving the skin of this area, while hemorrhagic fissures appeared in the dermis of the feet. The quantity of riboflavin in the ration did not appear to influence the time of onset or incidence of either dermatitis or perosis. The type of perosis present was dissimilar to that observed in poults receiving rations low in choline or manganese. The legs appeared weak and although the tendon of Achilles seldom slipped permanently from its condyle, the outward manifestation was a wobbly gait and a loose-joint appearance in the hock joints. The poults stood with their hocks further apart than normal and the angle formed by the tibia and metatarsus was increased until a 'peg-leg' appearance resulted.

The use of choline, biotin, and yeast fractions in the study of dermatitis and perosis

Following the experiments which showed that riboflavin did not influence the incidence of dermatitis, the work was extended to include the rôle of biotin, choline and various fractions of yeast in turkey nutrition. These results as related to dermatitis and perosis are recorded in table 3.

Poults receiving rations adequate in biotin but inadequate in any fraction of the aqueous extract of yeast or in riboflavin were protected from dermatitis but not from perosis. The alcohol-precipitate fraction when fed as the only vitamin concentrate added to the basal ration was ineffective in the prevention of perosis or dermatitis. A combination consisting of a biotin concentrate with either the ammonium hydroxide eluate or the alcohol-precipitate fraction was required to protect the poults from perosis. A ration containing 30 parts of untreated fresh egg white and 70 parts of a practical ration produced perosis and dermatitis in poults. The incidence of perosis did not always parallel the incidence of dermatitis in poults receiving rations deficient in biotin. This agrees with the report of Jukes and Bird ('42) that with chicks a smaller intake of biotin is required to prevent perosis than is required to prevent dermatitis. The above authors reported that 0.34 μg. of biotin per chick per day was enough to completely protect the chick from perosis but not enough for protection against dermatitis. We were unable to show that inositol either increased or decreased the incidence of perosis; however, choline, when fed at a level of 0.2% in addition to 10% strain G dried brewers' yeast[4], gave complete protection from perosis. A

TABLE 3

Effect of choline, biotin and yeast fractions on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 2[1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMATITIS	PEROSIS
		%	%
Strain G yeast			
Without choline	18	0	78
Plus 0.05% choline	12	0	33
Plus 0.10% choline	10	0	30
Plus 0.15% choline	9	0	11
Plus 0.20% choline	17	0	0
Basal only	8	100	50
Yeast residue	30	0	53
Without riboflavin	22	0	45
Plus aqueous extract of yeast	26	0	0
Plus norite eluate	18	0	0
Plus norite eluate plus inositol	17	0	0
Plus fuller's earth eluate	10	0	0
Plus inositol	16	0	56
Plus 1% salt mixture no. 30	17	0	47
Aqueous extract of yeast	10	60	0
Biotin II	6	0	50
Alcohol precipitate	31	84	71
Plus biotin I	10	0	0
Plus biotin II	10	0	0
Plus crystalline biotin methyl ester[2]	5	0	0
Aqueous extract of yeast			
Plus biotin I	11	0	0
Plus biotin II	12	0	0
Practical ration	10	0	0
Plus 30% raw egg white	10	70	60

[1] Basal ration 2 was composed of the simplified basal ration (table 1) plus 200 μg. Ca-pantothenate, 500 μg. riboflavin and 200 mg. choline per 100 gm. of ration, unless modified as indicated in the body of the table.

[2] Crystalline biotin methyl ester was fed by pipette at a level of 2.5 μg. on the 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, and 21 days of age.

---

[4] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.

combination of choline, biotin and a factor or factors which can be adsorbed on norite or fuller's earth and eluted with ammonium hydroxide appear to be required for prevention of perosis in turkey poults.

SUMMARY

1. Riboflavin did not influence the incidence of dermatitis in turkey poults.
2. Biotin was found to be an anti-perosis and an anti-dermatitis factor.
3. The anti-perosis property of choline was confirmed.
4. Inositol did not influence the incidence of perosis.
5. One or more factors present in an eluate from an adsorbate of an aqueous extract of dried brewers' yeast possessed anti-perosis properties.
6. It appears, therefore, that at least three organic factors are required to protect the poult from perosis, namely, choline, biotin and an unrecognized factor or factors which can be adsorbed on fuller's earth or norite and subsequently eluted with ammonium hydroxide.

LITERATURE CITED

ANSBACHER, S., AND M. LANDY 1941 Biotin and scaly dermatosis of the chick. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 3.

DU VIGNEAUD, V., K. HOFMANN, D. B. MELVILLE AND PAUL GYÖRGY 1941 The isolation of biotin (vitamin H) from liver. J. Biol. Chem., vol. 140, p. 643.

HEGSTED, D. M., R. C. MILLS, G. M. BRIGGS, C. A. ELVEHJEM AND E. B. HART 1942 Biotin in chick nutrition. J. Nutrition, vol. 23, p. 175.

HEGSTED, D. M., J. J. OLESON, R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1940 Studies on a dermatitis in chicks distinct from pantothenic acid deficiency. J. Nutrition, vol. 20, p. 599.

HOGAN, A. G., L. R. RICHARDSON, H. PATRICK AND H. L. KEMPSTER 1941 Perosis due to a vitamin deficiency. J. Nutrition, vol. 21, p. 327.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

JUKES, T. H. 1938 The vitamin G requirements of young poults. Poultry Sci., vol. 17, p. 227.

———— 1940 Effect of choline and other supplements on perosis. J. Nutrition, vol. 20, p. 445.

JUKES, T. H., AND F. H. BIRD 1942 Prevention of perosis by biotin. Proc. Soc. Exp. Biol. and Med., vol. 49, p. 231.

LEPKOVSKY, S., AND T. H. JUKES 1936 The response of rats, chicks and turkey poults to crystalline vitamin G (flavin). J. Nutrition, vol. 12, p. 515.

MCELROY, L. W., AND T. H. JUKES 1940 Formation of the anti egg-white-injury factor (biotin) in the rumen of the cow. Proc. Soc. Exp. Biol. and Med., vol. 45, p. 296.

PATRICK, H., R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL 1941 Biotin and prevention of dermatitis in turkey poults. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 456.

RECORD, P. R., AND R. M. BETHKE 1942 Further observations on choline and yeast in chick nutrition. Poultry Sci., vol. 21, p. 271.

RICHARDSON, L. R., AND A. G. HOGAN 1941 The anti-perosis complex. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 459.

WILGUS, H. S. JR., L. C. NORRIS AND G. F. HEUSER 1937 The role of manganese and certain other trace elements in the prevention of perosis. J. Nutrition, vol. 14, p. 155.

BULLETIN 449 SEPTEMBER 1943

Nutritive Requirements of Young Pigs

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

DIGEST

A ration composed of yellow corn, low protein tankage, alfalfa, and salt, which is adequate for pigs weighing approximately 75 pounds, was found to be inadequate for weanling pigs weighing about 30 pounds.

Weanling pigs fed this ration showed various gross symptoms of the deficiency including growth failure, lack of appetite, lesions of the skin, rough hair coats, and in some instances vomiting, scours, and stiffness.

The specific factor or factors lacking in this ration are not known. As far as growth was concerned, the deficiency was overcome almost completely by supplementing the ration with vacuum dried liver. There were unexplained death losses among the liver fed pigs, and survivors, although they grew well, sometimes were lame and affected with dermatitis.

The supplementary feeding of a combination of pure B complex vitamins was virtually ineffective in correcting the deficiency of the ration.

The ration was made practically complete by substituting various combinations of soybean oil meal and alfalfa leaf meal for part of the tankage, and by including in the ration various combinations of soybean oil meal, carotene, and yeast.

It is apparent that weanling pigs require unidentified nutritional factors which need not be supplied directly in the rations of older and heavier pigs.

Nutritive Requirements of Young Pigs*

R. C. Miller, T. B. Keith, W. T. S. Thorp and M. A. McCarty†

LOSSES OF YOUNG PIGS by death present a serious economic problem. The actual extent of these losses is unknown, but only 60 per cent of the annual pig crop reaches the market, according to estimates *(20)*. It is indicated that the mortality of pigs between the time of birth and weaning is at least 30 per cent *(17, 18, 22)*, and it is known that further losses occur after weaning. The losses of spring pigs seem to exceed those of fall pigs.

Management, infectious disease, and nutrition each appear to play a role either separately or together, in contributing to the well being of the nation's swine herd. In many instances it may be difficult to determine the exact function of these factors; for instance, whether a disorder is of an infectious or a nutritional origin, or if a nutritional deficiency predisposes infection. In any case the part played by nutrition cannot be arbitrarily discounted if all of the facts are to be considered.

In recent years there has been a noteworthy advance in the knowledge of the nutritive factors required by the pig. A requirement for adequate protein of good quality and for certain mineral elements, particularly calcium and phosphorus, is now presupposed. The need for adequate supplies of vitamins A and D is also now recognized. More recent evidence *(4, 8, 9, 10, 11, 12, 16, 21, 23, 24, and 25)* now shows that certain members of the vitamin B complex are definitely required by the pig.

While there is a need for further work to establish the exact requirement of the pig for certain of these nutrients, there is also much to be gained in studying how well the pig's nutritive requirements may be satisfied by commonly used feedstuffs.

Hogan and Johnson *(6 and 7)* reported observations on the inadequacy of concentrates commonly fed to brood sows. The feedstuffs used were corn, tankage, linseed oil meal, alfalfa meal, wheat bran, cod liver oil, dried buttermilk, skim milk, and minerals. The weaning weights of litters were subnormal and the mortality rate, 40 per cent, was excessively high. Abnormalities observed in the surviving pigs were skin lesions, muscular incoordination and weakness, diarrhea, and collapse. On post-mortem examination fatty livers, fatty kidneys, and hemorrhagic areas were found. It was concluded that the concentrates commonly used in swine rations were partially inadequate as the sole ration for the brood sow.

---

* Authorized for publication June 23, 1943.

† Associate Professor of Agricultural and Biological Chemistry, Assistant Professor of Animal Husbandry, Associate Professor of Animal Pathology Research, and Associate Professor of Animal Husbandry, respectively.

In the course of a study of the protein requirements of pigs *(14 and 15)*, the authors found that in many instances rations which were fully adequate for 50 to 75 pound pigs were wholly inadequate for weanling pigs weighing 25 to 35 pounds. In addition, frequently among groups of pigs which were thriving on a particular ration, one or two individuals failed to grow on the same ration.

A particular instance was that of 5 pigs fed a ration composed of yellow corn, 60.5 parts; 34 per cent protein tankage, 35 parts; ground alfalfa, 4 parts; salt, 0.5 part; and manganese sulfate, 0.02 part. This ration supported the growth of heavier pigs fairly well in previous tests; but when it was fed to a group of weanling pigs, two failed to grow at a normal rate, two died, and another, while it survived, did not grow but lost 5 pounds during 98 days of feeding. This pig was inactive, had spells of vomiting, suffered almost a complete loss of appetite and was affected with a mild dermatitis with lesions of the tail, legs, lips, and face. The three surviving pigs were given 50 milligrams of nicotinic acid, together with riboflavin and pyridoxine daily after the first 49 days. At one time, at three consecutive 3-day intervals, nicotinic acid was injected hypodermically. This treatment was apparently without effect, since two of the pigs died. The surviving pig was then given 100 grams of vacuum dried pork liver daily. During the ensuing 63 days this pig gained 53 pounds, the skin lesions disappeared, and the normal color and gloss of the skin and hair were restored, figs. 1 and 2. Similar observations were made at the same time with seven other individuals.

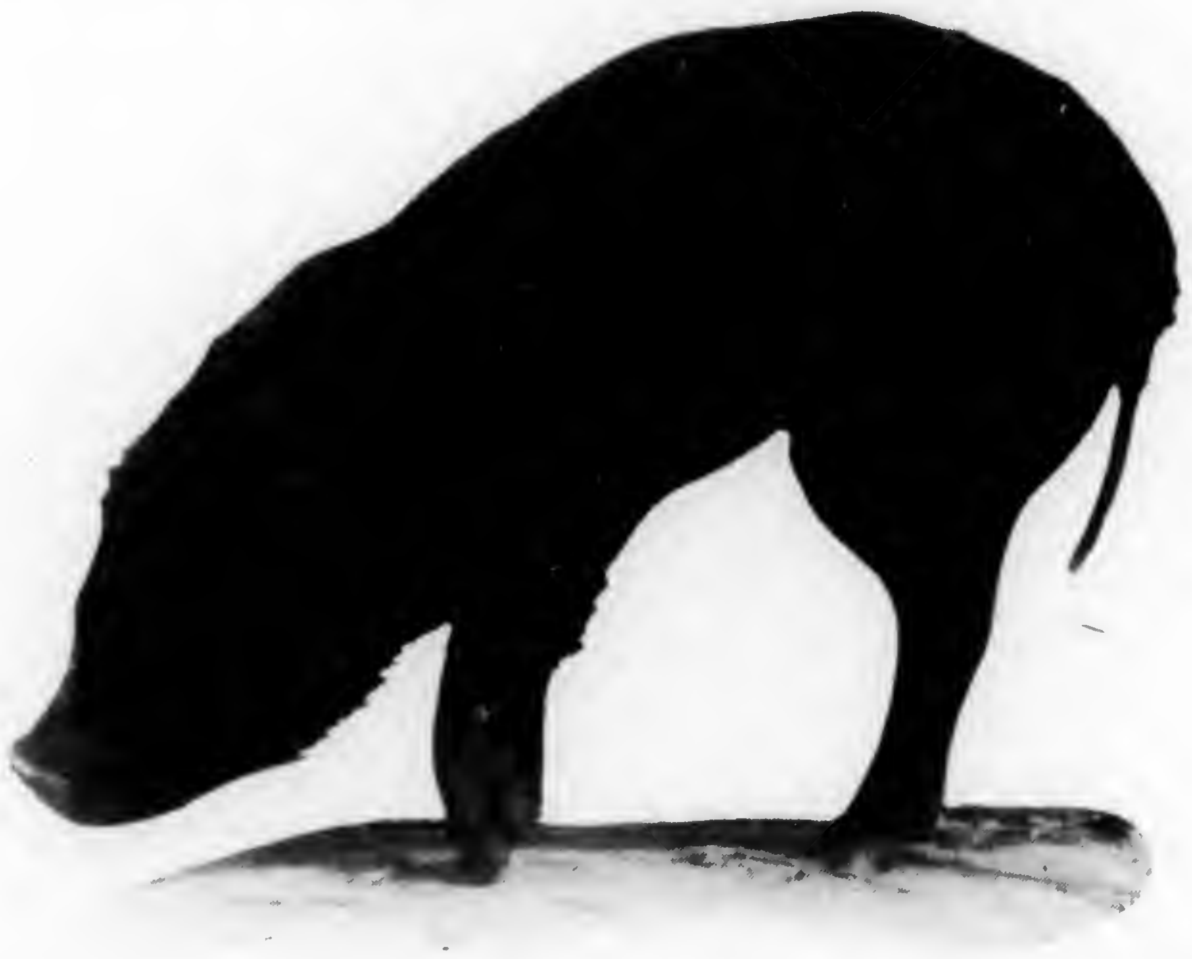


Fig. 1.—This pig weighed 26 pounds when placed on experiment. Ninety-eight days later, when the picture was taken, it weighed 21 pounds.

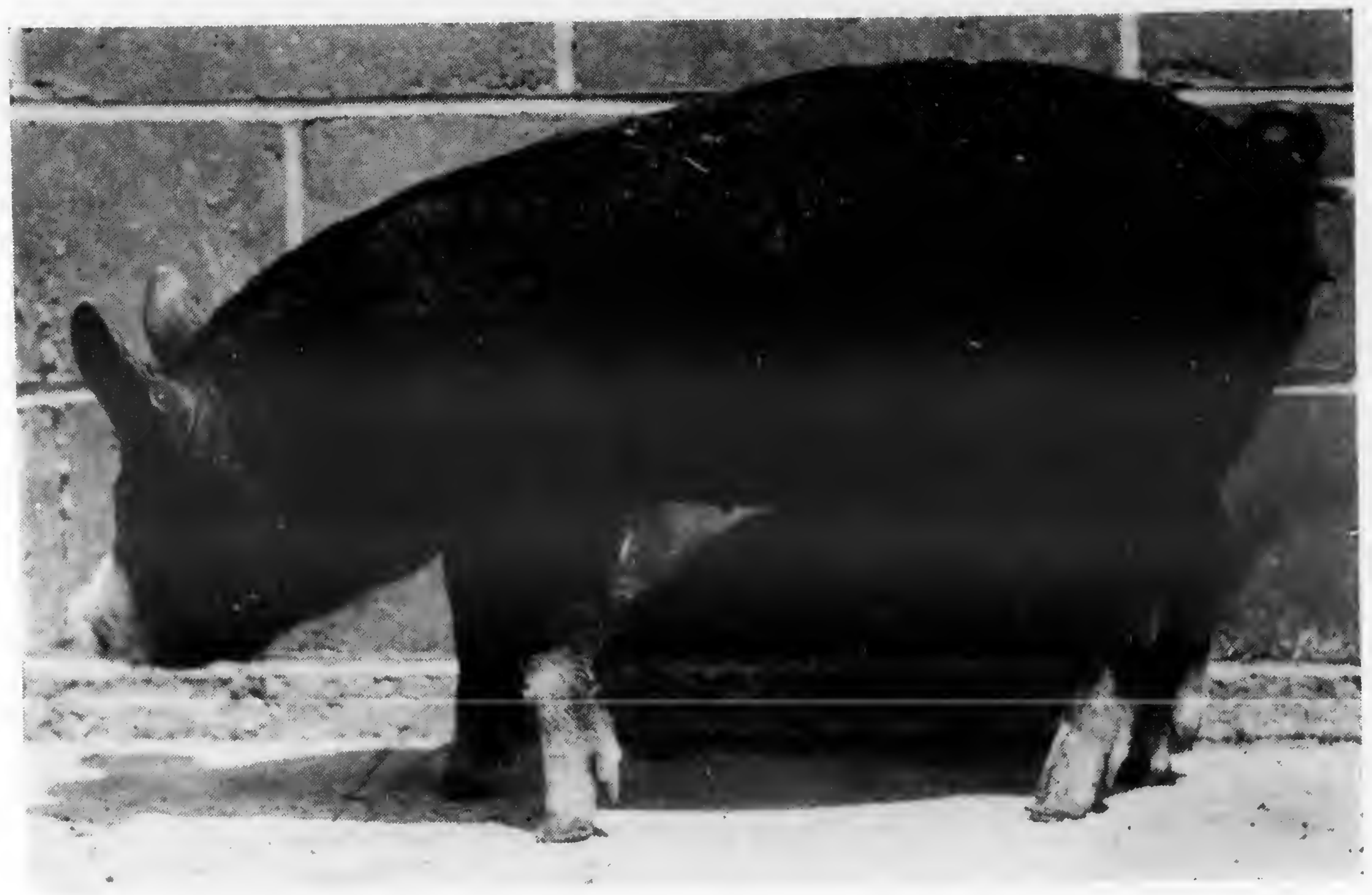


Fig. 2.—The same pig as shown in figure 1, weighed 74 pounds 105 days later. During this time the same ration as was fed previously was supplemented with daily feedings of vacuum dried liver.

It is perhaps not surprising that this ration is deficient for weanling pigs; nevertheless it is difficult to designate its deficiencies. In addition to growth failure the most noticeable outward symptoms shown by the pigs were abnormal skin coat, diarrhea, diminished activity, and lack of appetite. Other abnormalities, less frequently noted, were muscular inco-ordination and lameness, and vomiting.

EXPERIMENT I

Having made these preliminary observations, an experiment was set up to study further the nature of the deficiencies involved in rations of this type and means of correcting these deficiencies by the use of common feedstuffs. The rations used are given in table 1.

Five pigs were assigned to each ration. They were distributed equally, among the various groups, with respect to sex, age, weight and breed, and were fed individually. They were housed in a barn with a concrete floor, which provided access at all times to outside concrete runways and whatever sunlight was available.

Table 1.—Constituents of rations fed groups of weanling pigs to determine nutritive requirements; Experiment I.

FEEDS SUPPLIED	GROUP NUMBER 1	2	3	4	5	6	7	8	9
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Yellow Corn	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5
Soybean oil meal (expeller)	—	—	—	—	—	—	10.0	10.0	10.0
Tankage (34 per cent protein)	35.0	35.0	35.0	35.0	35.0	35.0	25.0	25.0	25.0
Ground alfalfa hay (sun cured)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vacuum dried whole beef liver	—	—	—	(b)	(c)	100 g. daily	—	—	—
Dried brewers' yeast	100 g. daily	(e)	—	—	—	—	(e)	100 g. daily	—
Carotene	(d)	40γ daily	(d)	—	—	—	(d)	—	40γ daily
Vitamin B factors	—	—	+ (a)	—	—	—	—	—	—

(a) Nicotinic acid 75 mg daily
Thiamin chloride 5 mg daily
Riboflavin 2 mg daily
Calcium pantothenate 10 mg daily
Pyridoxine 8 mg daily

(b) Liver extract added after 8 weeks
(c) Whole vacuum dried beef liver added after 8 weeks
(d) Carotene added after 8 weeks
(e) Brewers' yeast added after 8 weeks

The growth curves for the surviving pigs are given in figure 3. Data showing the losses of pigs by death are given in table 2.

On the basal ration, the results with groups 4 and 5 were similar to those obtained previously. At the end of 8 weeks all of these pigs were emaciated and weak. There was a general lack of appetite, some vomiting and diarrhea, and a mild dermatitis.

Histological examinations of the skins of pigs similarly affected with dermatitis were made on sections obtained at the time of post-mortem examination. On sections of the cutaneous lesions a diffuse cellular infiltration of the cutis was observed. In the early lesions a moderate hyperkeratosis was observed in some areas. In the areas of more extensive changes and hyperkeratosis, there was some edema of the cutis, while in several sections there were some small patchy areas of edema in the outer portion of the cutis. This change was accompanied by a few hyperemic and dilated blood vessels. In some sections small superficial epidermal abscesses and ulcerations were observed. In the more extensive areas of dermatitis there was an extensive crusting over of the surface, fig. 4.

After eight weeks on the basal ration three of these pigs (group 5, table 1) were given a daily supplement of 100 grams of whole vacuum dried beef liver, and the remaining two (group 4) were given an alcoholic extract of the same liver. There was an immediate response with both groups which, however, was more outstanding for the group fed whole liver. The latter regained a normal condition and made good growth throughout the remainder of the experiment. The recovery of the pigs fed liver extract was limited, but their growth and condition was improved to some extent.

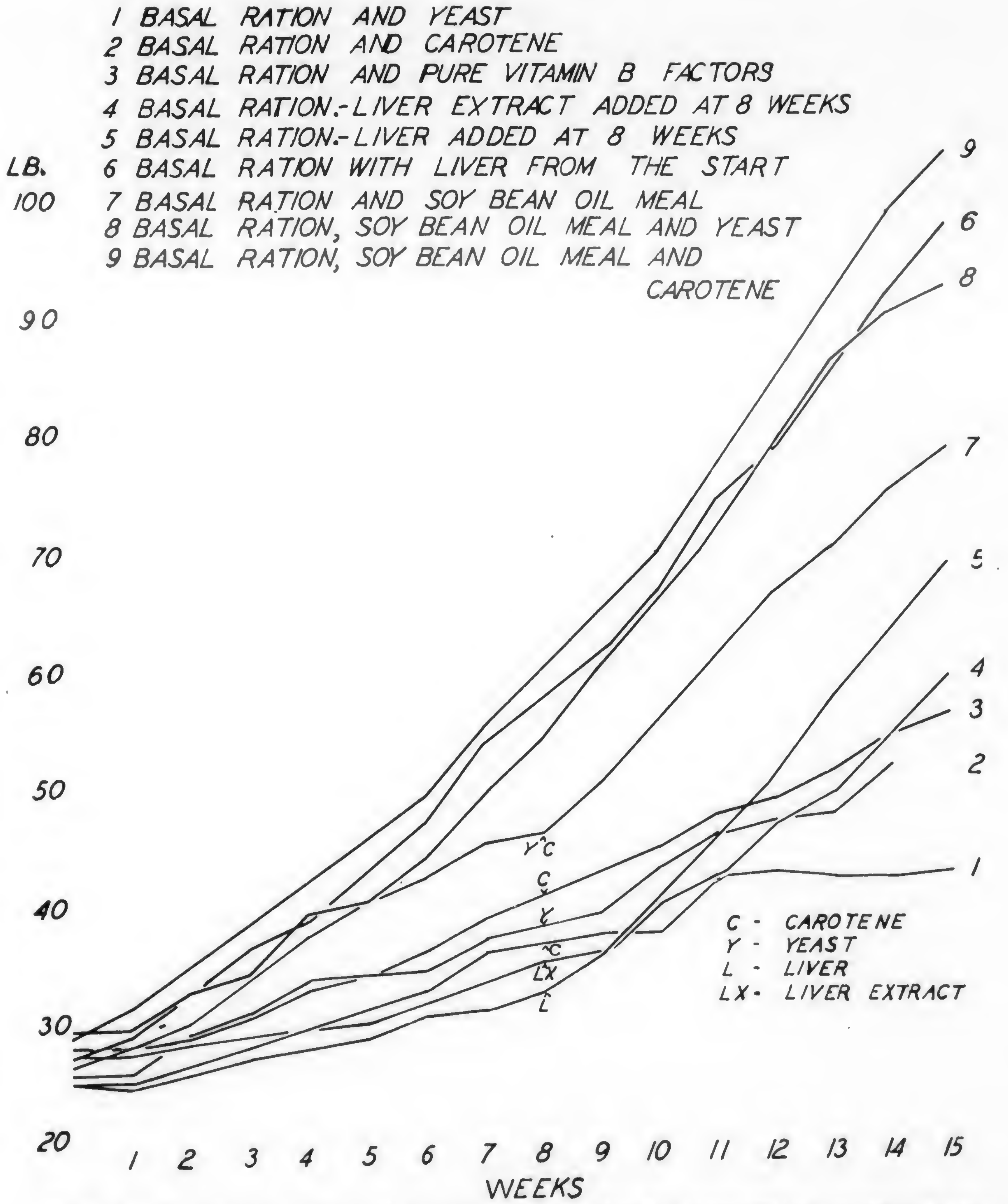


Fig. 3.—Growth curves of surviving pigs fed to determine nutritive requirements; Experiment I, table 2.

The results for the pigs fed the basal ration plus supplements of dried brewers' yeast (group 1), carotene (group 2), and vita-

Table 2.—Summary of death loss, clinical symptoms, and gross pathology of weanling pigs fed to determine nutritive requirements; Experiment I.*

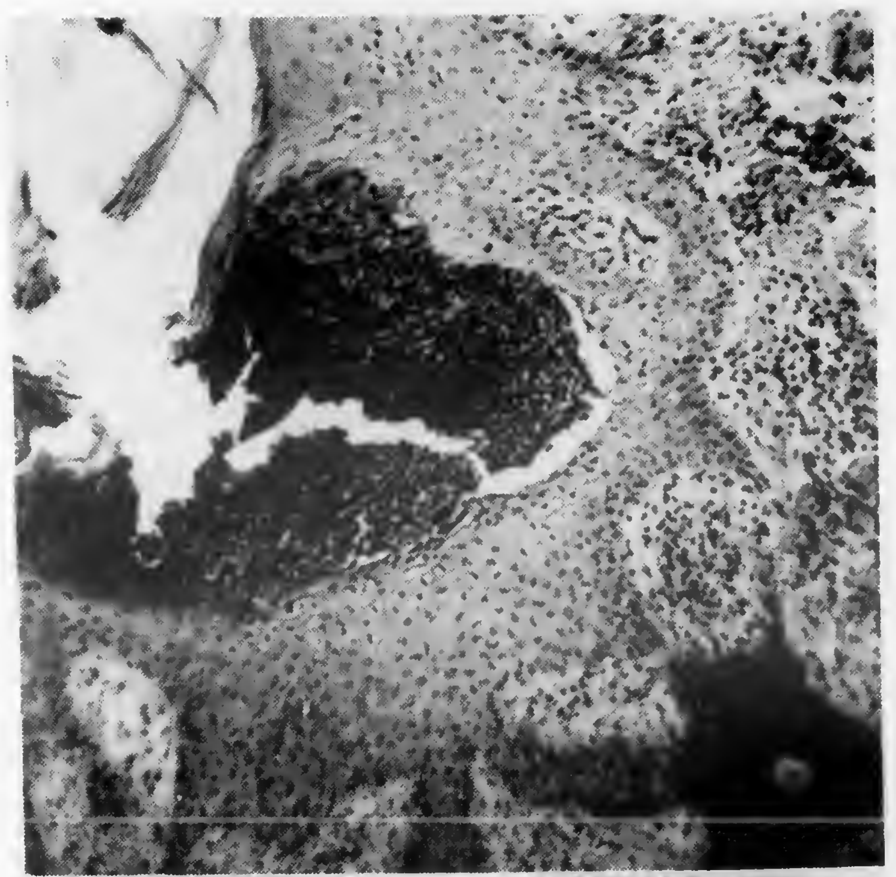
GROUP NUMBER	NUMBER OF PIGS STARTED ON EXPERIMENT	NUMBER OF PIGS THAT DIED	WEEKS OF EXPERIMENT IN WHICH DEATH OCCURRED	CLINICAL SYMPTOMS AND GROSS PATHOLOGY
1	5	3	4	This pig died suddenly. No gross pathological changes were found on post-mortem examination.
			5	This pig was killed for examination. No gross pathological changes were found.
			15	Previous to death this pig was unable to rise to its feet unless assisted. Its gait was unsteady; it appeared to be dizzy and walked with the head twisted. There was some swelling and inflammation of the conjunctiva and a mild dermatitis. In addition to the regular feed the pig had been given supplementary choline.
2	5	0		
3	5	2	3	This pig had some swelling over the shoulders with resulting stiffness. This condition cleared up when the pig was put on pasture, but death occurred after the pig was again placed on experimental feed.
			15	There was inflammation and swelling of the conjunctiva with partial impairment of the vision.
6	5	3	4	No gross pathological changes were found on post-mortem examination. There was a dermatitis in the ears causing them to be very sensitive.
			4	No gross pathological changes were found on post-mortem examination. There was mild dermatitis.
			5	There were diffuse subcutaneous hemorrhages on the loin and thigh. There were hemorrhages on the liver, spleen, and kidney which were accompanied by some scarring and grayish white mottling of the parenchyma.
7	5	2	9	This pig suddenly went off feed. It recovered its appetite when placed on grass but died after being placed on experimental feed.
			11	This pig died of sub-acute bronchopneumonia. There was mild dermatitis.

* There were no death losses in groups 4, 5, 8, and 9.

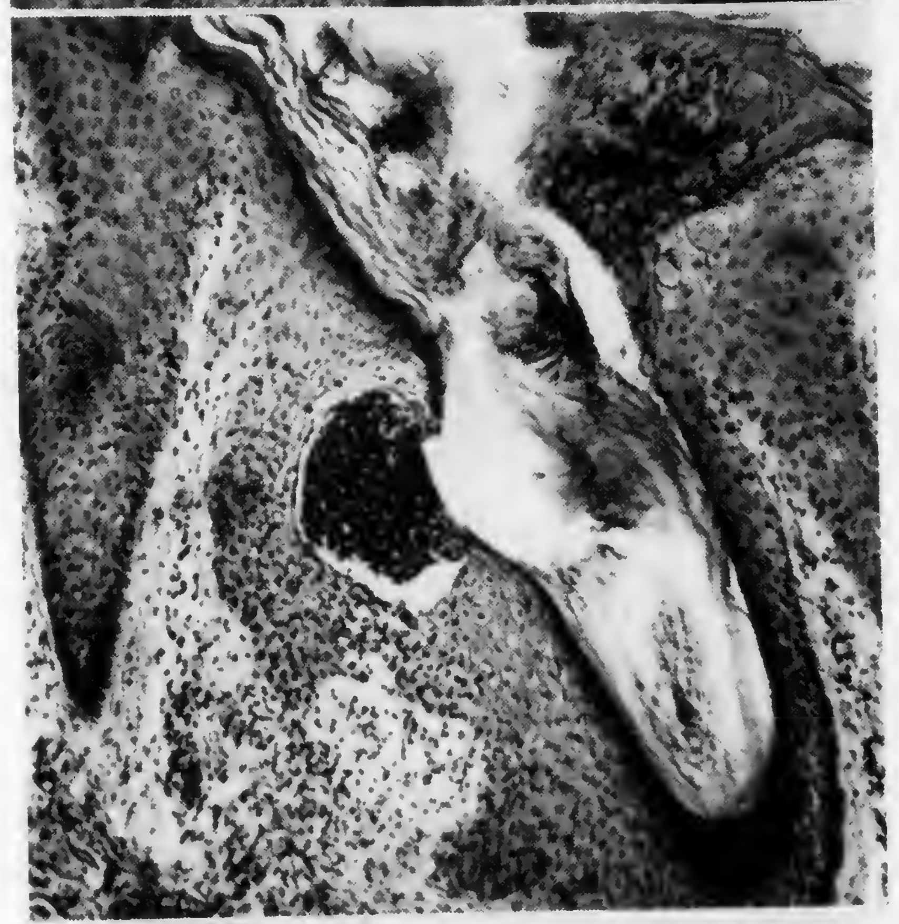
min B factors (group 3) were but slightly better than for the pigs on the basal ration, figs. 5 and 6. They showed the same gross symptoms, and three pigs died in each of the lots fed yeast and the B factors, respectively. In both cases two pigs died in the early weeks of the experiment and one died in the last week. At the end of 8 weeks of feeding, carotene was added as an additional supplement for the yeast and vitamin B groups, and yeast was given to the group receiving carotene. No striking responses were obtained, and the pigs continued to grow slowly.

Groups 7, 8, and 9 were fed the basal ration modified by substituting 10 per cent of expeller process soybean oil meal for 10 per cent of the tankage. In addition group 8 was given a daily

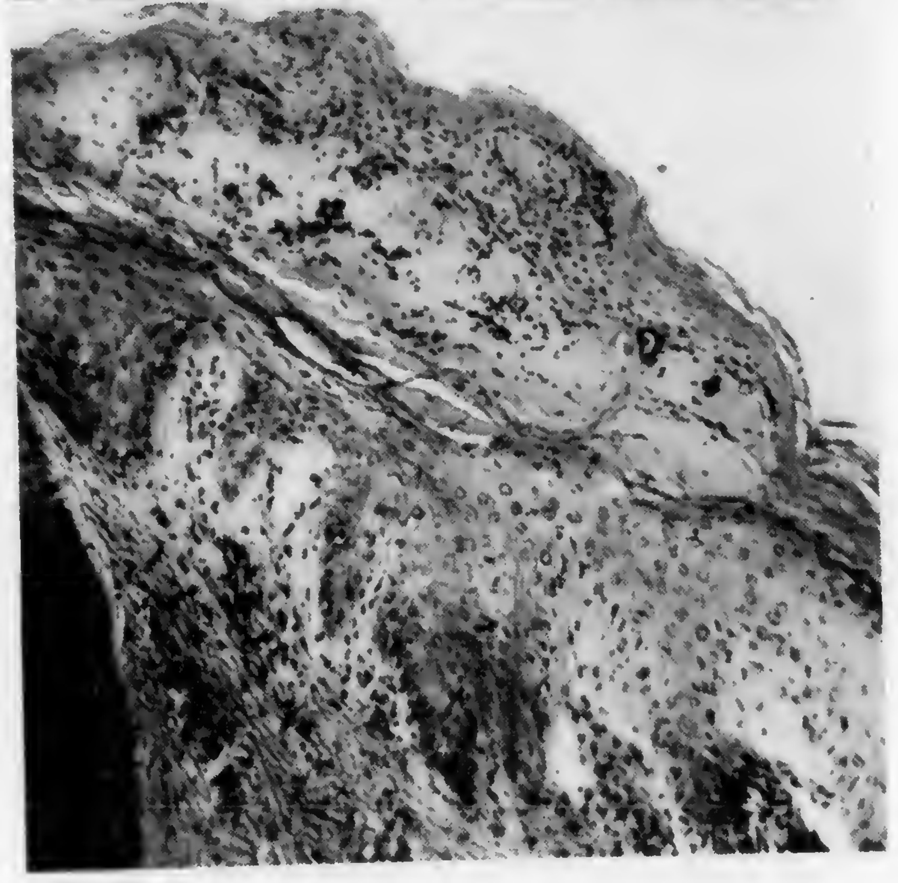
Figure 4.—



A.—Section of the skin showing a small superficial ulcerated area.



B.—Section of the skin showing an early cellular infiltration into the cutis and a small abscess next to a hair follicle.



C.—Section of the skin showing some edema of the cutis and a hyperkeratosis on the outer surface.

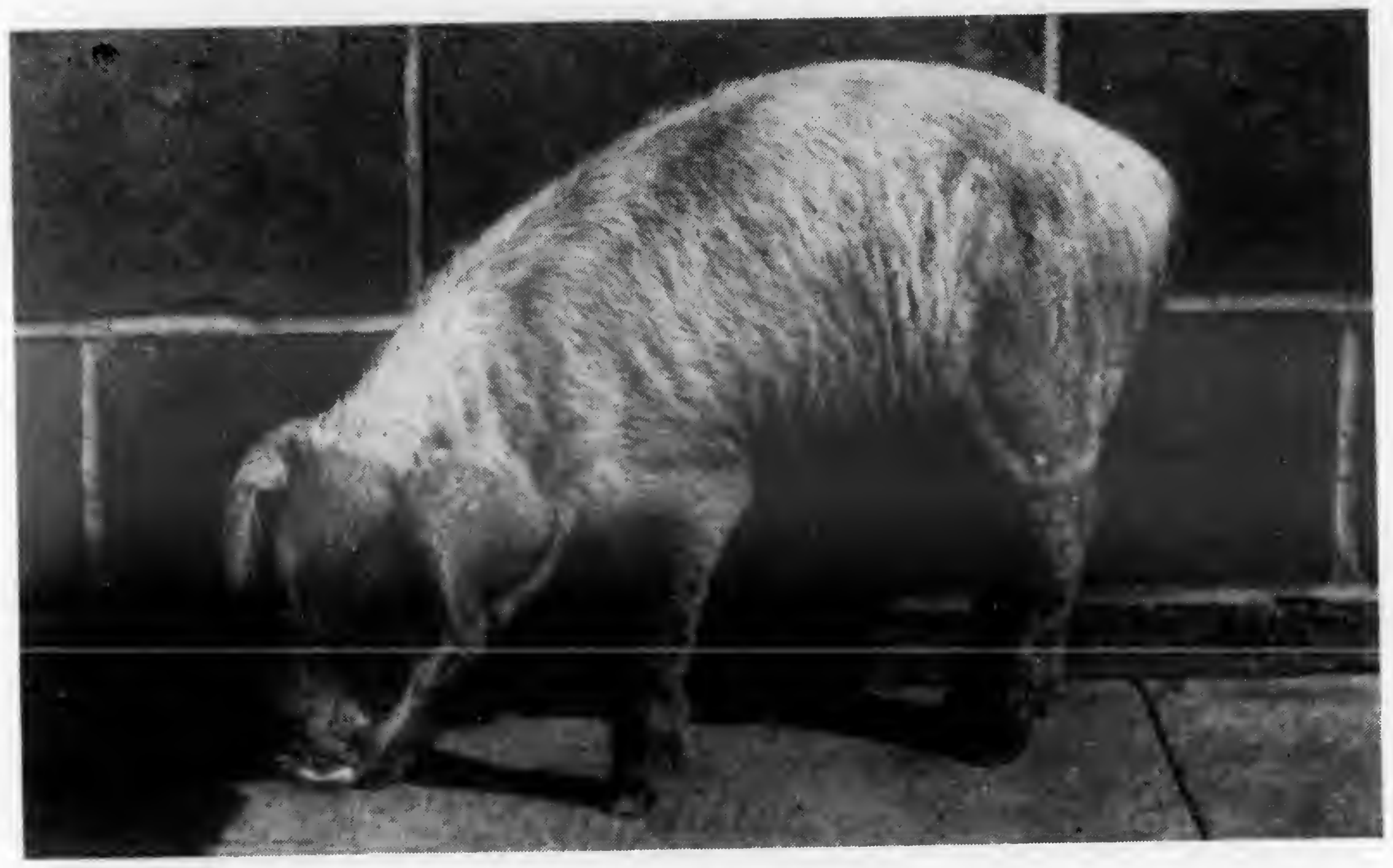


Fig. 5.—A Chester White barrow after being fed 105 days on the basal ration with 40 gammas of beta carotene daily.

supplement of brewers' yeast and group 9 was given carotene. Judging by the performance of the pigs of group 7, these supplements were effective in increasing the growth and improving the condition of the pigs. There was no mortality in groups 8 and 9

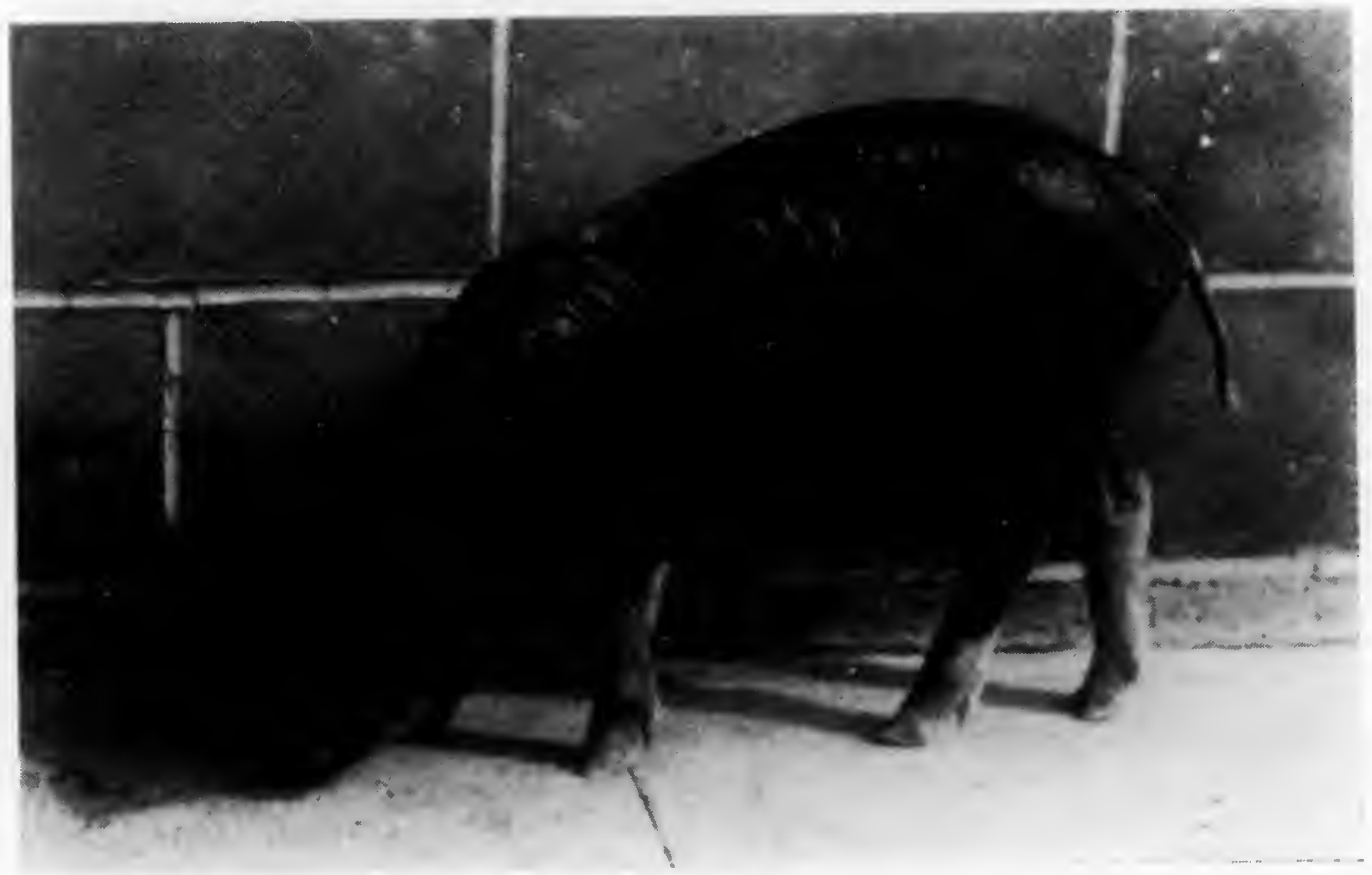


Fig 6.—A Berkshire gilt after being fed 105 days on the basal ration and 100 grams of yeast daily.

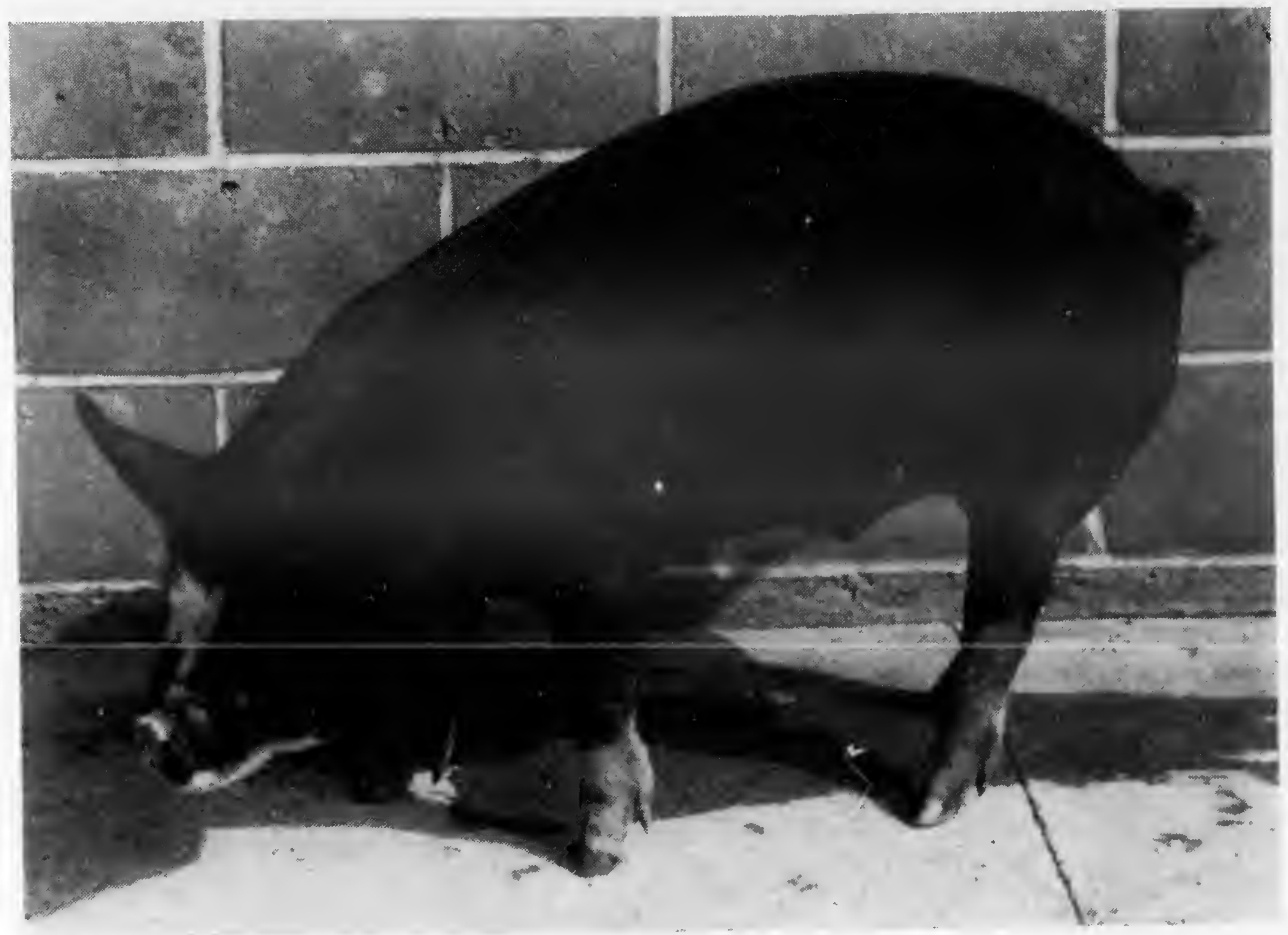


Fig. 7.—A Berkshire barrow after being fed the basal ration with 10 parts of the tankage replaced by expeller process soybean oil meal and 40 gammas of beta carotene daily for 105 days. Its coat was sleek and glossy, its skin was oily and healthy, and the animal was free from any lameness.

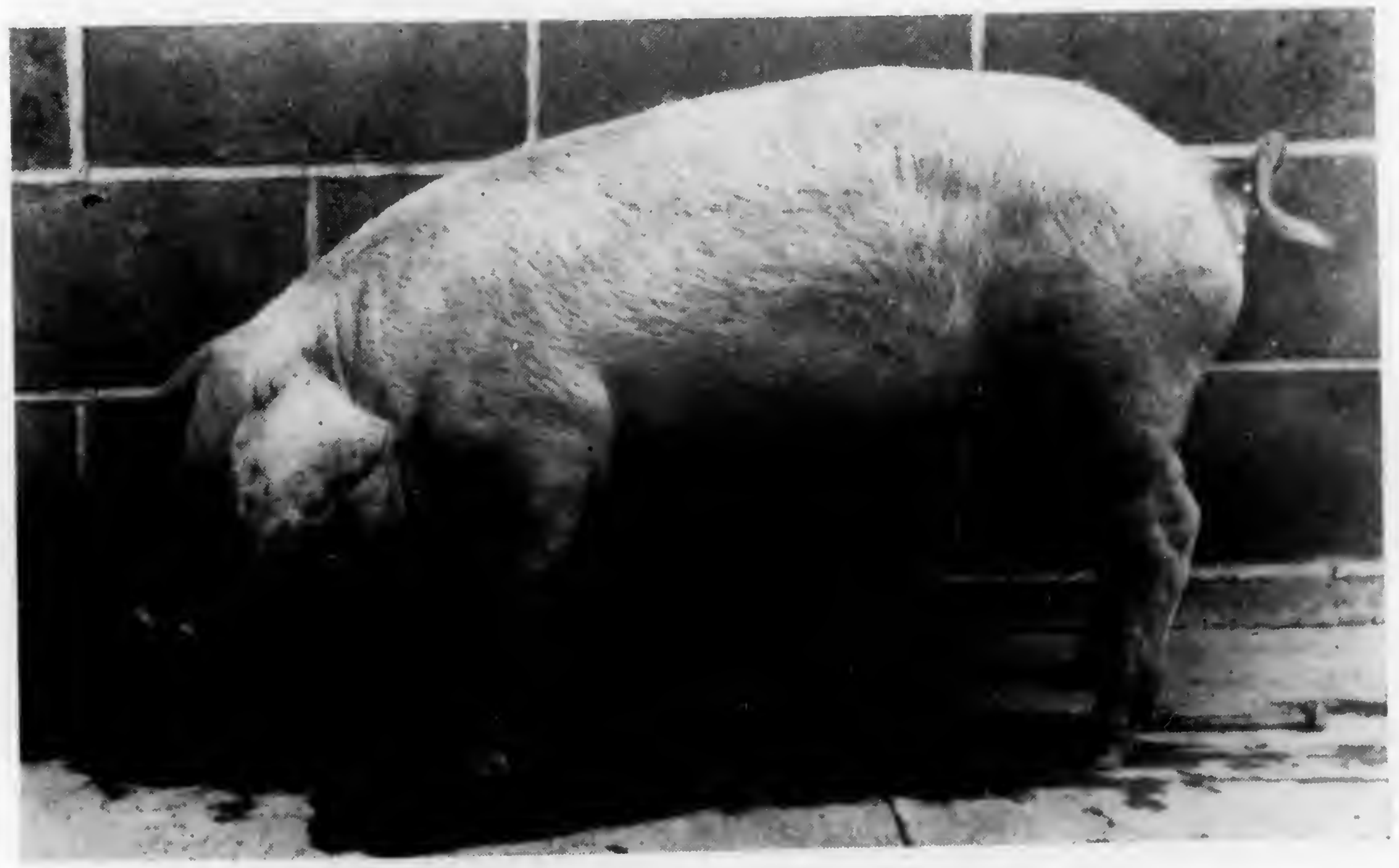


Fig. 8.—A Chester White barrow after being fed the same ration as the pig shown in figure 7. The pig was lame and a dermatitis was present on legs, tail, and ears.

while two pigs of group 7 died. The growth of the pigs fed soybean oil meal and carotene was the best of any in the experiment.

The pigs receiving 10 per cent of soybean oil meal instead of tankage made better growth than the pigs fed the basal ration alone but did not grow as well as the pigs receiving soybean oil meal with the carotene and yeast supplements. At the end of eight weeks, carotene and yeast were added to their ration with a resulting favorable response.

All of the pigs of group 9 were normal with respect to skin condition and gait and had the healthy appearance of the pig shown in figure 7, except the one in figure 8. This pig made an average daily gain of 0.7 pounds but had a moderate dermatitis, and ataxia developed at about 105 days. After feeding on alfalfa pasture for three weeks the ataxia became more pronounced, and within a short time the animal was unable to walk, fig. 9.

One pig of group 8 had a moderate dermatitis and diarrhea and became slightly lame after 105 days of feeding.

The pigs of group 6 received the basal ration with the liver supplement throughout the experiment. During the first five weeks, three of these pigs died as the result of internal hemorrhages the cause of which was undetermined. The growth of the two remaining pigs was uniformly good and was of the same order as that of the pigs which received soybean oil meal with the yeast and carotene supplement (groups 7, 8, and 9). However, at the end of 105 days of feeding the skins of both pigs were dry and scaly. The hair coats were dull. Both pigs eventually became somewhat lame and moved with difficulty, figs. 10, 11, and 12. The lameness did not become severe; however, it was not alleviated when these pigs were placed on alfalfa pasture for five weeks at the end of the regular experimental feeding period.

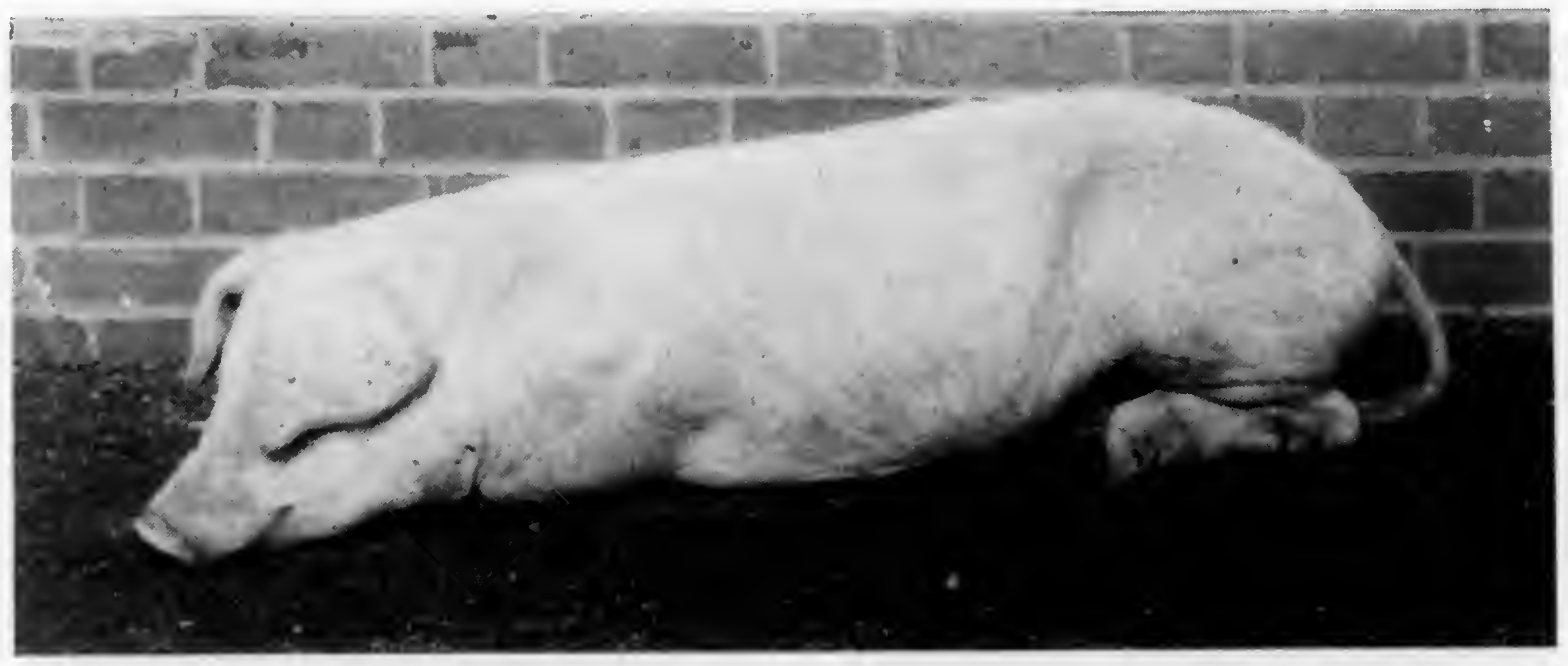


Fig. 9.—The pig shown in Fig. 8 after a subsequent three weeks on alfalfa pasture. At this time the pig could not stand. Large necrotic areas were present on hocks, legs, and tail.

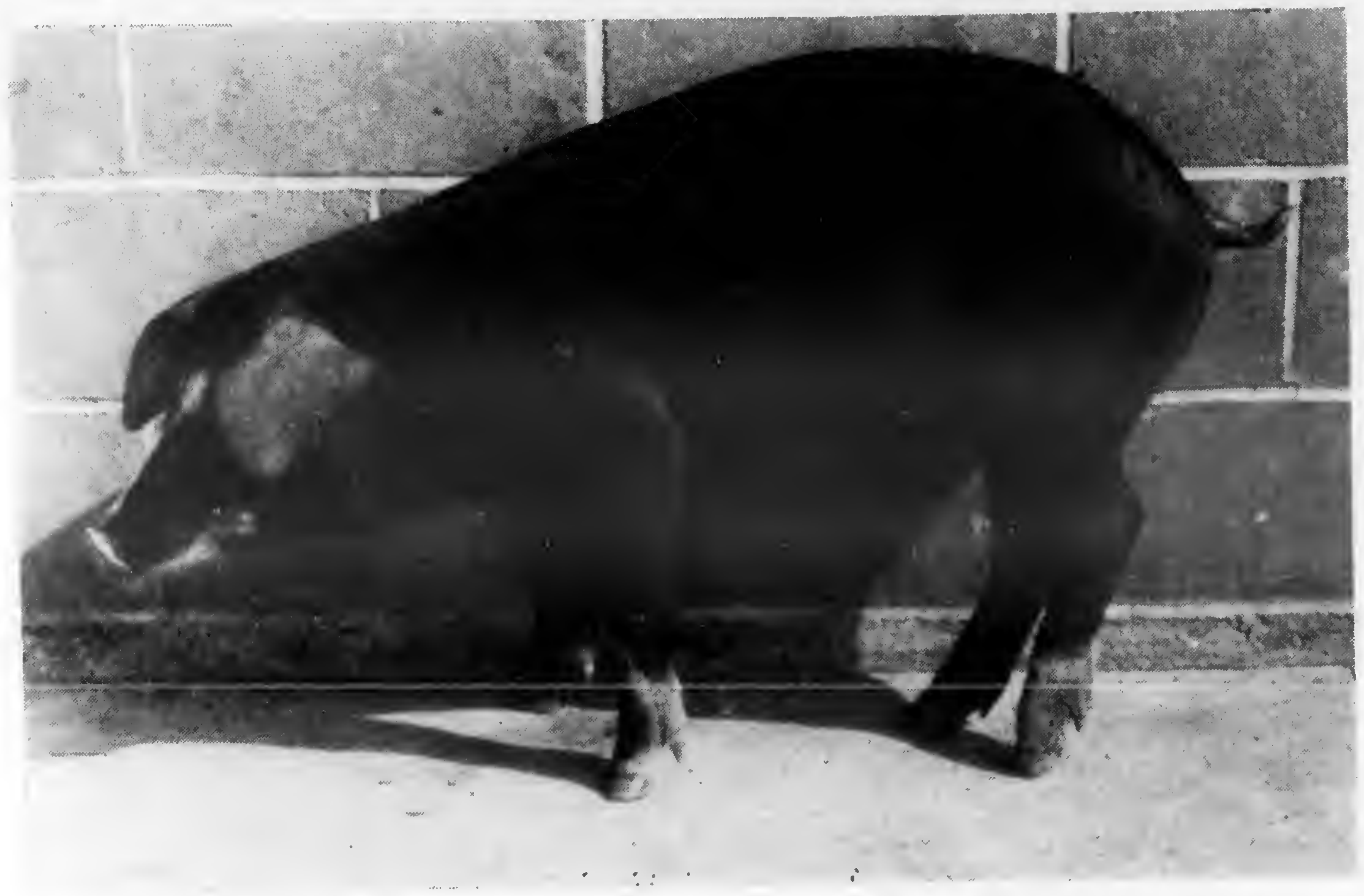


Fig. 10.—A Poland China barrow after being fed 105 days on the basal mixture with 100 grams of vacuum dried beef liver daily. The animal was lame and a slight dermatosis was present on its legs.

The results of this experiment showed that vacuum dried whole beef liver apparently contained a substance or substances which were deficient in the basal ration. The substitution of 10 per cent of soybean oil meal for 10 per cent of the tankage in the basal ration and adding supplemental carotene or yeast was also effective in overcoming the deficiency.

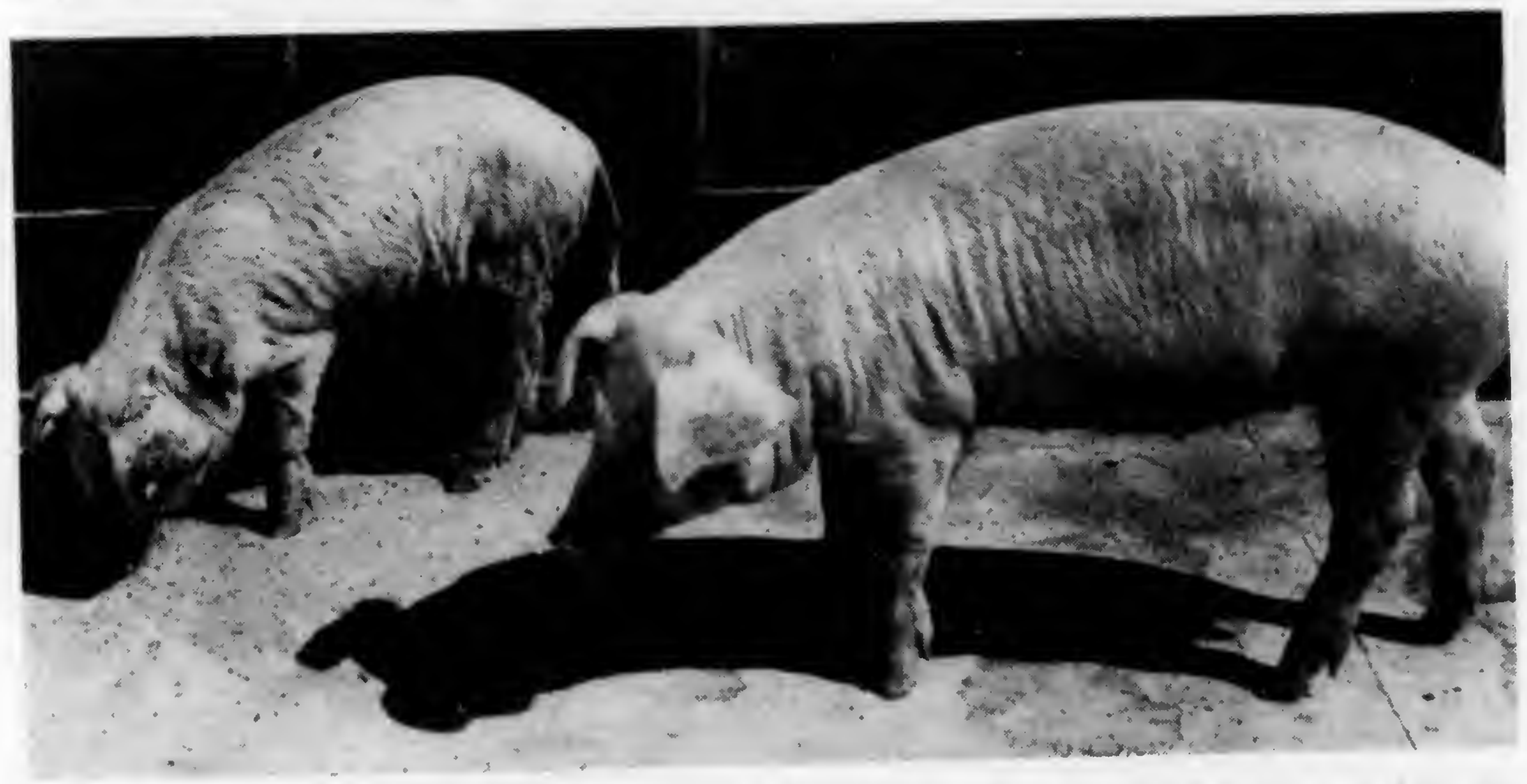


Fig. 11.—Two pigs after 84 days of feeding. The smaller pig was fed the basal ration and 40 gammas of beta carotene daily. The larger pig was fed the basal ration and 100 grams of vacuum dried beef liver daily. The larger pig had made normal growth, but later became lame and had a slight dermatitis.

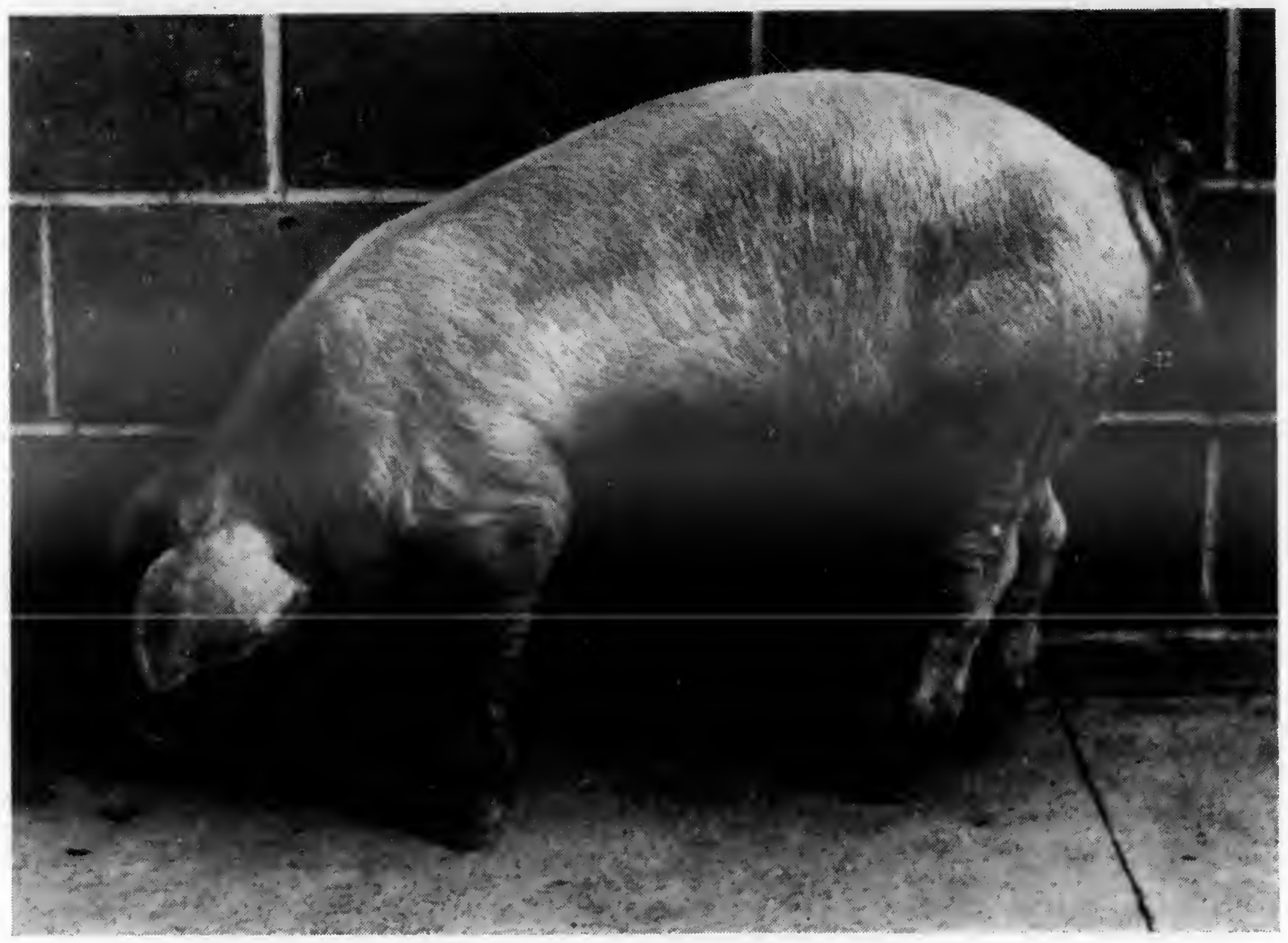


Fig. 12.—The Chester White gilt shown in figure 11 after being fed for 105 days on the basal mixture plus 100 grams of vacuum dried beef liver. The pig was slightly lame and had a dermatitis.

EXPERIMENT II

Subsequently another experiment was planned in which various combinations of soybean oil meal and dehydrated alfalfa meal were used in modifying the original basal ration, table 3, fig. 13.

Table 3.—Constituents of rations fed groups of weanling pigs to dermine nutritive requirements; Experiment II.

FEEDS SUPPLIED	GROUP NUMBER 1	2	3	4	5	6	7	8
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Yellow Corn	54.3	54.3	54.3	65.3	78.3	78.3	59.3	59.3
Tankage (34 per cent protein)	15.0	15.0	15.0	30.0	—	—	20.0	20.0
Tankage (60 per cent protein)	—	—	—	—	17.0	17.0	—	—
Soybean oil meal (expeller)	20.0	20.0	—	—	—	—	10.0	10.0
Soybean oil meal (solvent)	—	—	20.0	—	—	—	—	—
Alfalfa leaf meal*	10.0	10.0	10.0	—	—	—	10.0	10.0
Alfalfa hay	—	—	—	4.0	4.0	4.0	—	—
Salt (NaCl)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Manganese sulfate ($MnSO_4$)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Dried brewers' yeast	—	+	—	—	—	—	—	+

* The alfalfa leaf meal was donated by the Denver Alfalfa Milling and Products Company.

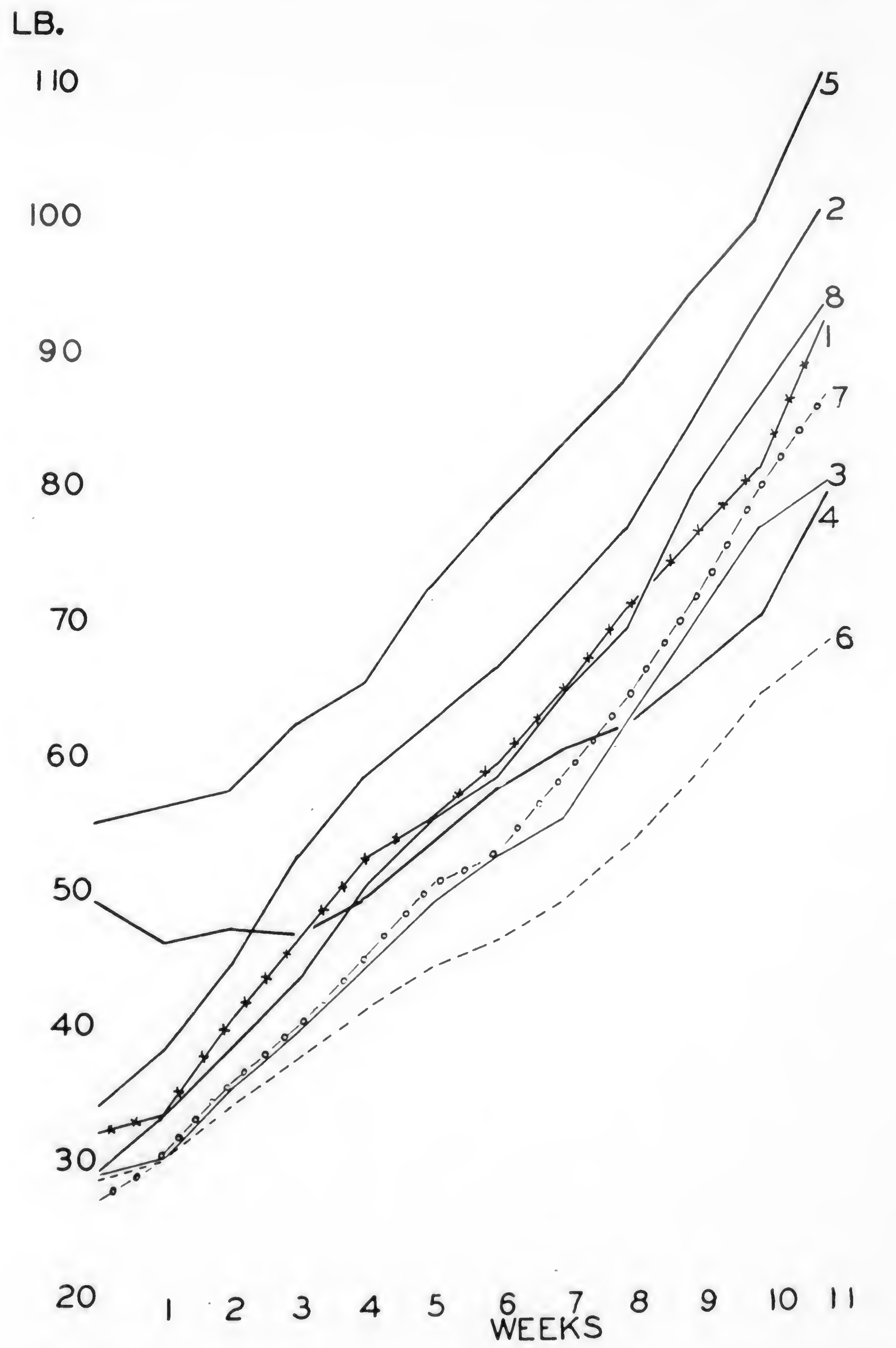


Fig. 13.—Growth curves of pigs fed to determine nutritive requirements; Experiment II, table 4.

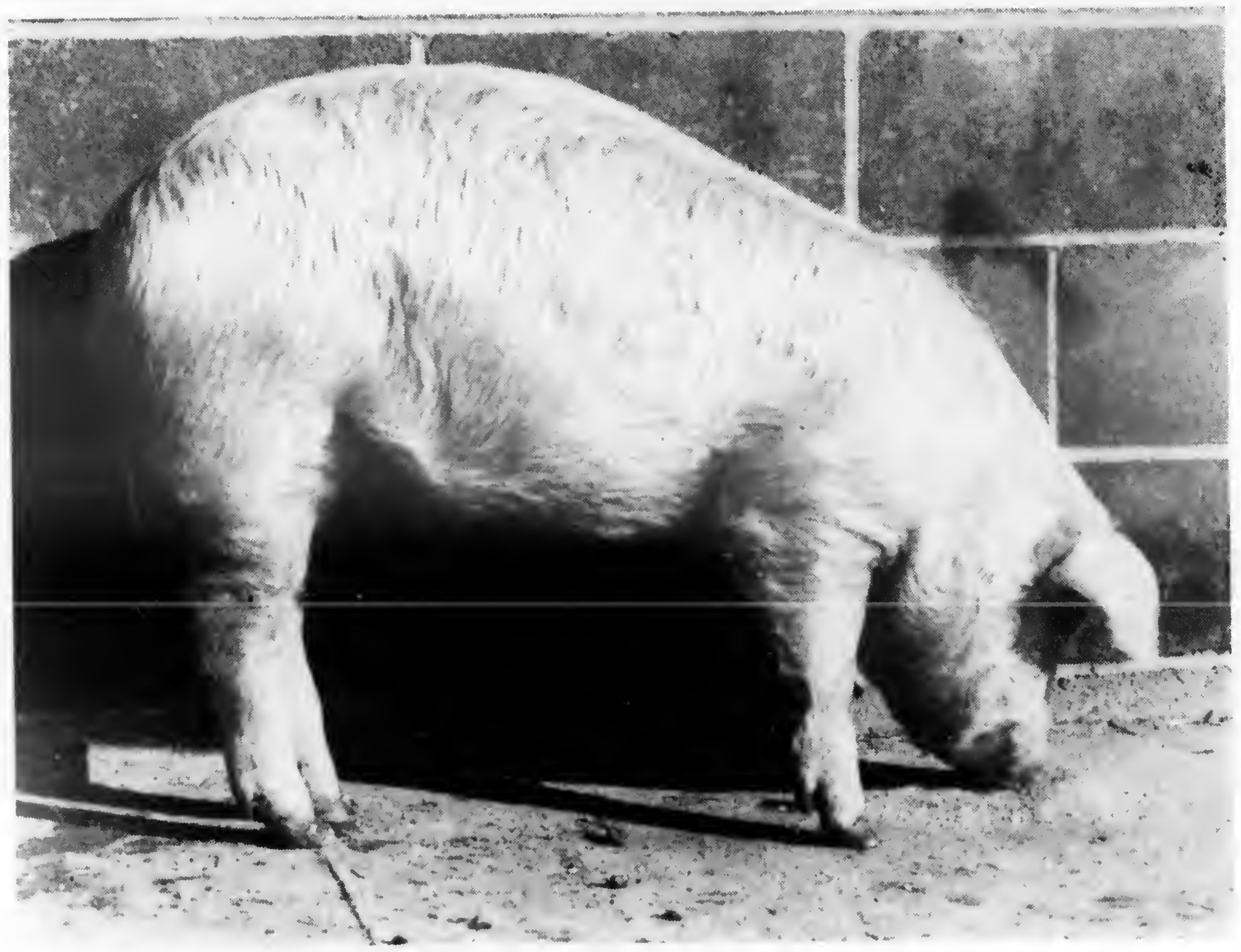


Fig. 14.—A Chester White sow from group 1, after being fed for 77 days. The animal was normal in every respect.

In groups 1 and 2, twenty per cent of expeller process soybean oilmeal was used to replace an equal amount of the tankage of the basal ration used in Experiment I, and 10 per cent of dehydrated alfalfa leaf meal was used instead of 4 per cent of sun-cured alfalfa hay. This combination appeared to give excellent growth, fig. 14, which in 11 weeks was equal to that of the best rations in Experiment I when pigs were fed for 15 weeks. Group 2 received a supplement of 100 grams of dried brewers' yeast daily which appeared to supplement the ration to some extent, the growth of the pigs of group 1 not being quite equal to that of the pigs of group 2, which received the yeast.

Rations fed groups 7 and 8 contained only 10 per cent of expeller soybean oil meal instead of 10 per cent of the tankage, and 10 per cent of dehydrated alfalfa leaf meal as in rations fed groups 1 and 2. Yeast was given, in addition, to the pigs of group 8, and the resulting growth was about the same as of groups 1 and 2. Without yeast (group 7) growth was slightly less.

The ration fed group 3 contained 20 per cent of solvent process soybean oil meal instead of the expeller process meal. The resulting growth with the solvent meal was of a lower order than on the expeller meal; nevertheless the solvent meal ration appeared not to be grossly deficient.

Table 4.—Gains, feed consumption, and economy of gains of pigs fed to determine nutritive requirements; Experiment II.

Group Number	Average Daily Gain	Average Daily Feed	Feed per 100 Pounds Gain
	pounds	*pounds*	*pounds*
2	0.86	2.48	249
8	0.84	2.26	265
1	0.78	1.99	287
7	0.65	1.82	294
3	0.65	1.87	292
6	0.50	1.63	302

The rations fed groups 5 and 6 were designed to be similar to the basal ration used in Experiment II except for the source of tankage, 60 per cent protein digester tankage being used in Experiment II instead of low protein tankage. Weanling pigs were able to grow slowly on this ration (group 6), but the growth was not so good as on the rations containing the combination of soybean oil meal and low protein tankage. When pigs with an initial weight of 55 pounds were fed this ration (group 5), the resulting growth was comparable to that obtained with the soybean oil meal rations and was much better than that of the pigs having an initial weight of 49 pounds which were fed the ration containing low protein tankage (group 4). In view of the authors' past work with the protein requirements of pigs *(14 and 15)* it is not felt that the different results obtained on low protein tankage and 60 per cent protein tankage can be explained on the basis of the quality or quantity of the protein in these products.

DISCUSSION

It is of particular interest that the results of these experiments show that the deficiencies of the ration composed of corn, low protein tankage, alfalfa, and salt may be corrected by combinations of soybean oil meal, carotene, and yeast as well as by liver. The slightly more favorable effect of 20 per cent over 10 per cent of soybean oil meal is shown in Experiment II. At the higher level of soybean oil meal feeding, the supplemental effect of yeast is not shown. In Experiment II the inclusion of 10 per cent of dehydrated alfalfa leaf meal undoubtedly supplied additional carotene which may have been lacking in some of the rations in Experiment I. Carotene apparently was not the only essential lacking in these rations; however, with at least 60 per cent of yellow corn in all of the rations it is surprising that carotene is involved in the deficiency of these rations.

The results obtained with the 60 per cent protein digester tankage in Experiment II are of interest, since they show that for the young pig this product is superior to low protein tankage, although the ration in which 60 per cent tankage was used is still not complete.

There is only one comparison, in these experiments, of solvent with expeller process soybean oil meal. The results for the most part are similar, but the solvent meal does not appear to be quite so good as the expeller product.

The gross symptoms shown by the deficient pigs in these experiments are somewhat similar to those given by Davis, Freeman, and Madsen *(2)* for pigs affected with necrotic enteritis. A further point of similarity to the results obtained by these workers is the excellent response to liver feeding. It has not appeared to the authors, however, that necrotic enteritis has been a factor in the present experiments. Furthermore, the feeding of nicotinic acid and of yeast were without favorable influence for the most part. However, liver feeding was always beneficial in restoring the growth and improving the condition of the pigs.

Breed *(1)* differentiates between infectious swine enteritis and nutritional enteritis. He states that the latter type of enteritis may develop as a result of the dietary lack of the specific vitamins, or as the result of impairment of the ability of the pig to assimilate and utilize the vitamin when it is present in the feed. This is due, he believes, to a pathological condition existing in the intestinal tract.

A further factor influencing the occurrence of dietary deficiencies is suggested by some of the results of these experiments, and by observations of numerous other cases made by the authors. An intrinsic factor is suggested which has to do with the quantity of food eaten by the individual. With certain rations which have been fully adequate for practically all individuals, nutritive failure has occurred with a few. When this type of failure occurs it is invariably found that the food consumption of the affected pig is lower than normal. In each instance it has not only been a question of a decrease in food consumption, but rather it is a case of the pig never consuming enough nutrients to permit normal development. Resulting growth is poor and the resistance of the animal is lowered. A change in the regime, supplying nutritive elements which are lacking, or stimulating to the appetite, results in a resumption of growth, in many instances, on the same ration.

The results obtained by feeding liver also suggest a similarity to the findings of Dunlop *(3)*, who suggested that liver contains large amounts of physin, an appetite-stimulating, growth promoting factor which is effective for pigs as well as for laboratory animals.

There have been a number of reports of the beneficial effects of feeding liver to pigs. Wintrobe, Miller and Lisco *(25)*, and Ellis and Madsen *(4)*, reported that liver is very effective in affording protection to pigs against a disorder characterized by degeneration in the nervous system and manifested by inco-ordination in the use of the legs.

Johnson and Palmer *(13)* also noted the beneficial effects of feeding liver, although they differentiated between a "so-called" growth factor and an appetite factor and attributed the increased growth obtained with rats fed various kinds of liver to appetite stimulation.

It is apparent that liver may contain some factor or factors as yet unidentified. Shaffer, McKibbin, and Elvehjem *(19)* have reported that there is present in liver extract a factor required by dogs which, with the possible exception of biotin, is not one of the currently recognized members of the vitamin B complex. In the absence of this factor, growth is usually arrested and anorexia and loss of weight occur.

A summary of the gains, food consumption and economy of gain for pigs on Experiment II are given in table 4. There is a direct correlation between the gains and the feed intakes. It is shown further that those rations producing the best growth were the most economical with respect to the amount of feed required per 100 pounds of gain. The feed intakes in this experiment were not restricted except by the choice of the pigs, due presumably to the inadequacies of some of the rations. The resulting amounts of feed required per 100 pounds of gain, therefore, reflect the completeness of the rations. According to Ellis and Zeller *(5)* restricting the feed allowance, on presumably adequate rations, generally decreases the quantity of feed required to produce a unit of gain. The results obtained in the present experiments indicate that with the increased food intake obtained on the more adequate rations, less feed is required per unit of gain. Thus the economy of gain also reflects the completeness of the ration.

BIBLIOGRAPHY

(1) Breed, F. 1942. Swine Enteritis. *The North American Veterinarian.* **23**: 79-83.

(2) Davis, G. K., V. A. Freeman and L. L. Madsen. 1940. The Relation of Nutrition to the Development of Necrotic Enteritis in Swine. *Mich. Agr. Sta. Tech. Bul.* **170**.

(3) Dunlop, G. 1935. The Effect of the Growth-Promoting, Appetite-Stimulating or "Physin" Factor on the Live Weight Increase of Swine. *Jour. Agr. Sci.* **25**: 445-459.

(4) Ellis, N. R. and L. L. Madsen. 1941. Relation of Diet of Swine to Development of Locomotor Inco-ordination Resulting from Nerve Degeneration. *Jour. Agr. Res.* **62**: 303-316.

(5) Ellis, N. R. and J. H. Zeller. 1934. Effect of Quantity and Kinds of Feed on Economy of Gains and Body Composition of Hogs. *U.S.D.A. Tech. Bul.* **413**.

(6) Hogan, A. G. and S. R. Johnson. 1940. Inadequacy of the Concentrates Commonly Used in Swine Feeding. *Missouri Agr. Exp. Sta. Res. Bul.* **321**.

(7) ———— 1941. Supplementary Value of Various Feedstuffs in Brood Sow Rations. *Missouri Agr. Exp. Sta. Res. Bul.* **332**.

(8) Hughes, E. H. 1938. The Vitamin-B Complex as Related to Growth and Metabolism in the Pig. *Hilgardia* **11**: 595-612.

(9) ———— 1939. The Role of Riboflavin and Other Factors of the Vitamin-B Complex in the Nutrition of the Pig. *Journ. Nutr.* **17**: 527-533.

(10) ———— 1939. The Minimum Requirement of Riboflavin for the Growing Pig. *Jour. Nutr.* **20**: 233-238.

(11) ———— 1939. The Minimum Requirement of Thiamin for the Growing Pig. *Jour. Nutr.* **20**: 239-241.

(12) ———— 1942. Pantothenic Acid in the Nutrition of the Pig. *Jour. Agr. Res.* **64**: 185-189.

(13) Johnson, D. W. and L. S. Palmer. 1934. The Appetite Stimulating and Growth Promoting Property of Liver. *Jour. Nutr.* **8**: 285-294.

(14) Keith, T. B. and R. C. Miller. 1940. Levels of Protein for Pigs. *Penna. Agr. Exp. Sta. Bul.* **401**.

(15) Miller, R. C. and T. B. Keith. 1941. The Optimum Level of Protein Intake for the Growth and Fattening of Swine. *Jour. Nutr.* **21**: 419-429.

(16) Patek, A. J., J. Post and J. Victor. 1941. Riboflavin Deficiency in the Pig. *Amer. Jour. Physiol.* **133**: 47-55.

(17) Phillips, R. W. and J. H. Zeller. 1941. Some Factors Affecting Fertility in Swine. *Amer. Jour. Vet. Res.* **2**: 439-442.

(18) Progress Report. 1941. *U.S.D.A. Bur. An. Ind. and Co-operating States, Regional Res. Lab. for the Improvement of Swine Through Breeding.*

(19) Shaffer, A. F., J. M. McKibbin and C. A. Elvehjem. 1942. Studies on the Vitamin B Complex in the Nutrition of the Dog. *Jour. Nutr.* **23**: 491-500.

(20) Smith, W. W. 1937. Pork Production. *The MacMillan Co.*

(21) Van Etten, C., N. R. Ellis and L. L. Madsen. 1940. Studies on the Thiamin Requirement of Young Swine. *Jour. Nutr.* **20**: 607-625.

(22) Wilcox, R. H., W. E. Carroll and T. G. Hornung. 1933. Some Important Factors Affecting Costs in Hog Production. *Ill. Agr. Exp. Sta. Bul.* **390**.

(23) Wintrobe, M. M., D. M. Mitchell and L. C. Kolb. 1938. Sensory Neuron Degeneration in Vitamin Deficiency. *Jour. Exp. Med.* **68**: 207-219.

(24) Wintrobe, M. M. 1938. Nutritive Requirements of Young Pigs. *Amer. Jour. Physiol.* **126**: 375-387.

(25) Wintrobe, M. M., J. L. Miller and H. Lisco. 1940. Relation of the Diet to the Occurrence of Ataxia and Degeneration in the Nervous System of Pigs. *Bul. Johns Hopkins Hosp.* **67**: 377-405.

Reprinted from Proceedings of the
AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE
Vol. 42, 1943

The Influence of Date of Sampling on the Value of Leaf Weights and Chemical Analyses in Nutrition Experiments with Apple Trees[1]

By DONALD E. H. FREAR and R. D. ANTHONY, *Pennsylvania Agricultural Experiment Station, State College, Pa.*

THE nutritional experiment with apple trees grown in metal cylinders recently completed at this station afforded a unique opportunity to study the interrelationships between chemical analyses of portions of the trees, physical measurements of the same trees, and their fertilizer treatment. The detailed reports of this experiment have been prepared, and will be published shortly. It is the purpose of this paper to present a study of the time of leaf sampling and its relation to the physical and chemical measurements made on these leaves, specifically the leaf weights and the nitrogen analyses of the leaves.

EXPERIMENTAL PLAN

Trees and Treatments:—The trees used in this experiment were of the Stayman Winesap variety, budded on Malling XII stock. Metal cylinders, 5 feet in diameter and 5½ feet deep, were set into the ground so that the upper edge extended approximately 6 inches above the surface of the surrounding earth. The bottoms of the cylinders were closed with concrete, allowing a drainage opening in the center, and were filled with Hagerstown silty clay soil dug and placed in the cylinders in three layers: surface 0 to 9 inches, subsurface 9 to 18 inches, and subsoil 18 to 48 inches. Forty-two of these cylinders were arranged in six rows of seven columns each, and one tree was planted in each cylinder in the spring of 1928. During 1928 and 1929 all of the trees received a uniform treatment of adequate amounts of nitrogen, phosphorus and potassium.

Beginning with the growing season of 1930, differential treatments were applied. For convenience the rows were designated by letters, the columns by figures. Row A received no inorganic nitrogenous fertilizer; row B, one unit; row C, two units, and so on, row F (the last) receiving five units of nitrogen. During subsequent years the amounts and chemical nature of the nitrogenous fertilizers applied were varied, but the relations between the rows was not. The columns, running from 1 to 7, received increasing amounts of green manure applications, column 1, none; column 2, one unit; column 3, two units, and so on, through column 7, which received six units of green manure. Thus each of the 42 trees in the experiment received a different treatment ranging from tree A-1 which received neither nitrogen nor green manure through tree F-7 which received five units of nitrogenous fertilizer and six units of green manure. Each tree in the experiment received uniform amounts of potassium and phosphorus in the form of inorganic fertilizers in amounts considered to be adequate for the size of the trees. The trees were grown in the cylinders until the autumn of 1938, at which time they were removed, weighed and sampled.

---

[1] Authorized for publication on January 29, 1943 as Paper No. 1163 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Sampling:—Several times during each growing season from 1932 through 1938 leaf samples were taken from a point midway on the terminal growth. No more than one leaf was taken from a terminal at any given sampling, and in most cases only one leaf was taken from a terminal per season. Usually four samples were taken, the first during late May or early June, the second during July or August, the third usually in September, and the fourth near the end of the growing season, usually in October. The exact dates of sampling and the number of leaves taken are given in Table I.

TABLE I—DATES OF SAMPLING AND NUMBER OF LEAVES TAKEN FROM EACH TREE

Year	Date	Number of Leaves
1932	May 18	36
1932	June 17	25
1932	August 2	20
1932	September 22	20
1933	June 29	20
1933	August 3	15
1933	September 1	15
1933	October 3	15
1934	June 4	20
1934	July 6	20
1934	August 2	20
1934	August 31	15
1934	October 17	15
1935	May 27	80*
1935	July 8	100*
1935	August 23	30
1935	October 3	30
1936	June 4	100*
1936	July 8	100*
1936	August 24	30
1936	October 2	40
1937	June 8	100
1937	July 13	80*
1937	August 24	30
1937	October 6	30
1938	June 15	100*
1938	August 18	100*
1938	September 26	100†

*Samples of the entire one year (terminal) growth collected and all leaves removed for analysis. The average number of leaves is indicated.

†Final sample taken at the time the trees were removed from soil. Two samples—spur leaves as well as terminal leaves—were collected at this time.

The leaves were taken to the laboratory immediately after collection, and were weighed after removal of the petioles. They were then dried in an air oven at approximately 80 degrees C until thoroughly dry, and were then removed, allowed to come to equilibrium with respect to the moisture in the air, and were again weighed and ground for analysis.

EXPERIMENTAL RESULTS

Only a portion of the experimental results will be given in this paper, those having a bearing on the relation between sampling date, chemical analyses and fertilizer treatment. More complete information on other phases of the experiment may be found in the detailed reports presented elsewhere[2] at a later date.

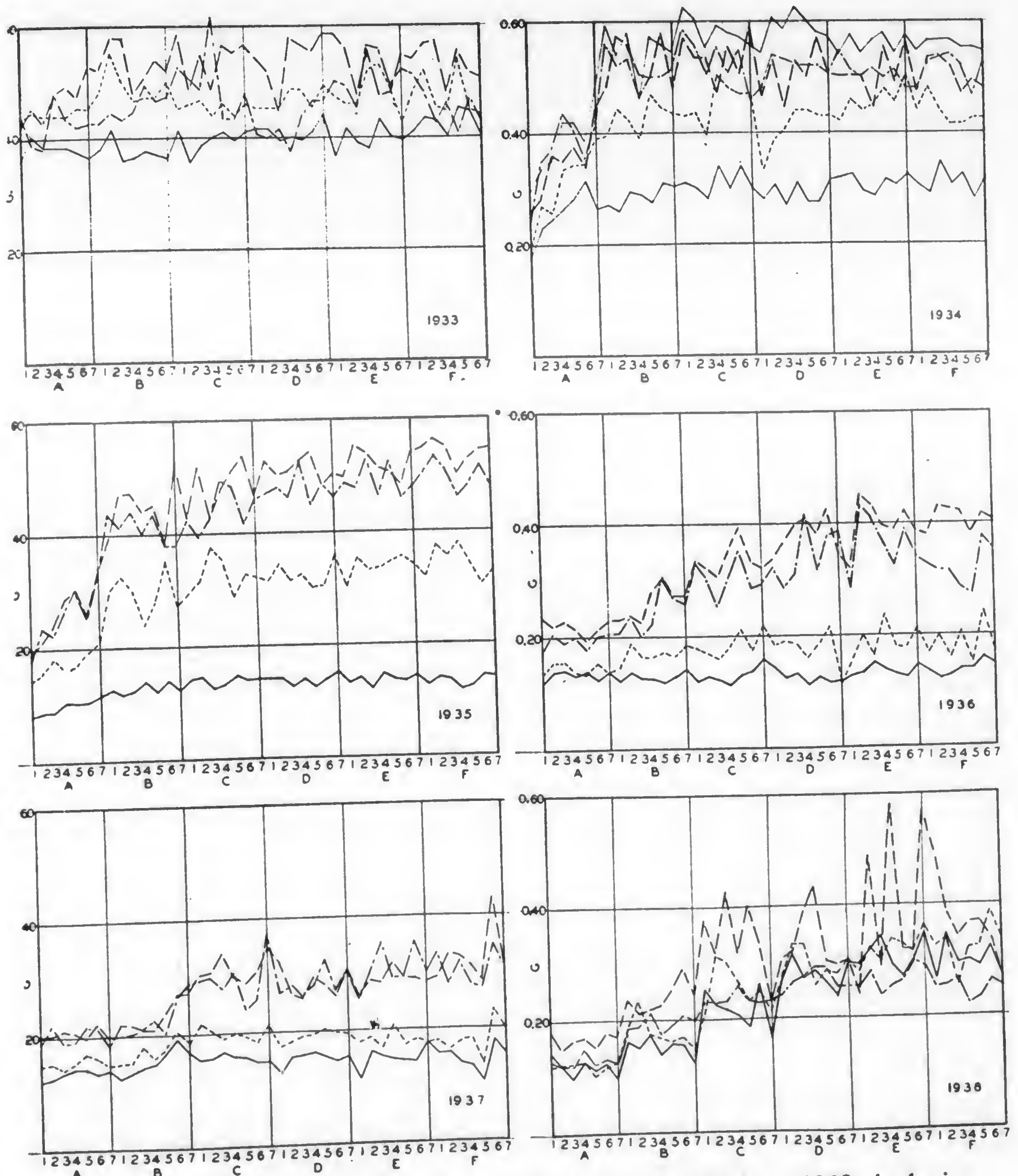


FIG. 1. Air-dry leaf weights for all samples taken, 1933 to 1938, inclusive.

Leaf Weights:—The air-dry leaf weights for all of the samples taken during the years 1933 to 1938 inclusive are given in Fig. 1. In each of the charts in this figure the solid line represents the first sampling, the short dotted line the second, the long dotted line the third, and the alternately long and short dotted line the fourth sampling. In 1934 a fifth sample was taken, represented by a lighter solid line.

---

[2] Response of Stayman apple trees in metal cylinders to varying amounts of inorganic nitrogenous fertilizers and green manures.

Part I. The influence of the various treatments on tree performance and the physical condition of the soil. Anthony, R. D., Fagan, F. N., and Clarke, W. S., Jr.

Part II. Chemical studies on the nitrogenous metabolism. Frear, D. E. H.

Part III. Chemical studies on the phosphorus metabolism. Frear, D. E. H.

Part IV. Chemical studies on the potassium metabolism. Frear, D. E. H.

Part V. Soil studies. Richer, A. C., and White, J. W.

Leaf Nitrogen:—For comparison with the leaf weight data the amounts of nitrogen present in the leaf samples are presented in Figs. 2 and 3. In Fig. 2 the amounts of nitrogen are expressed as percentages of the air-dry leaf weights; while in Fig. 3 the amounts are expressed as milligrams of the element per leaf.

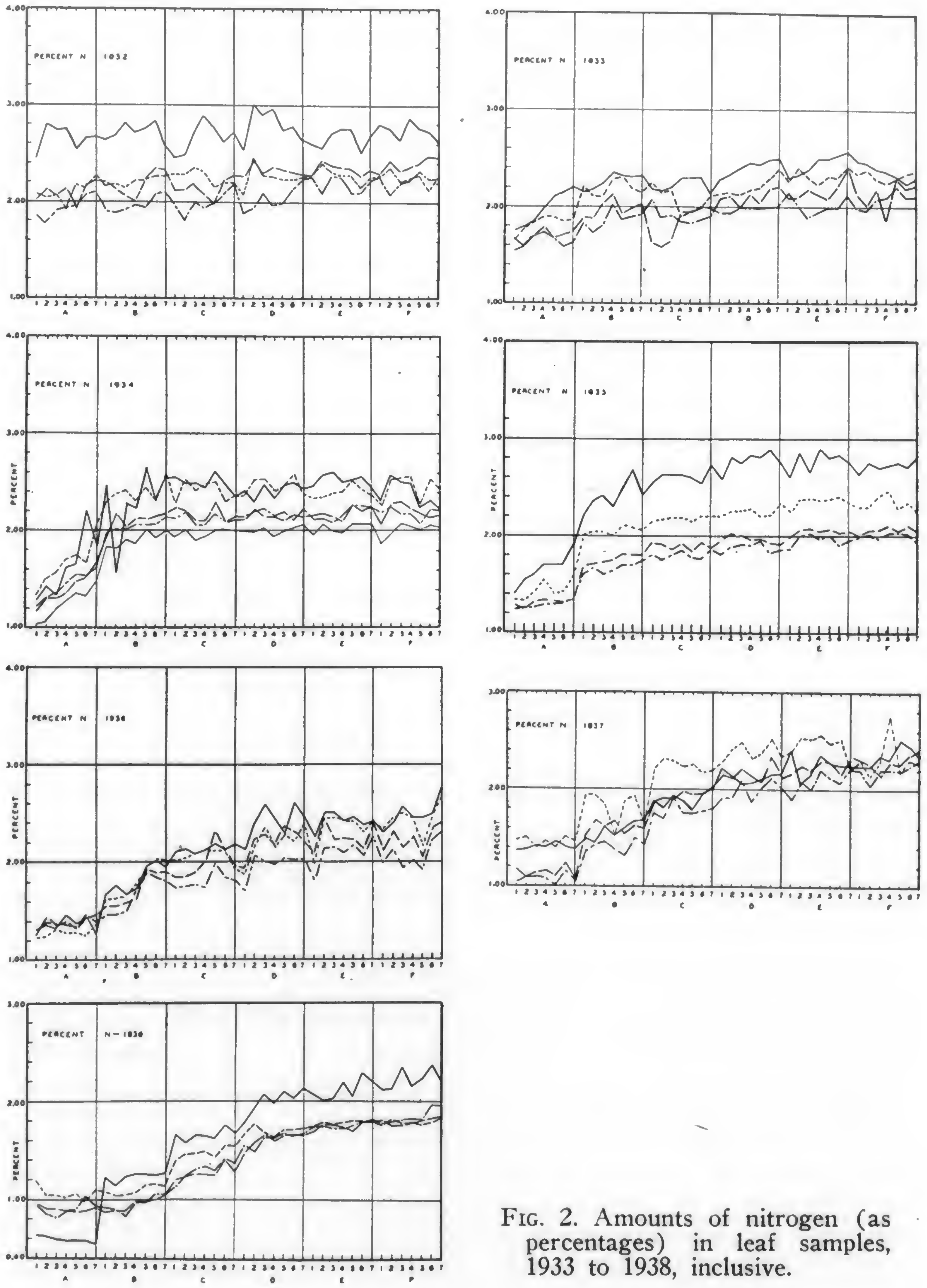


FIG. 2. Amounts of nitrogen (as percentages) in leaf samples, 1933 to 1938, inclusive.

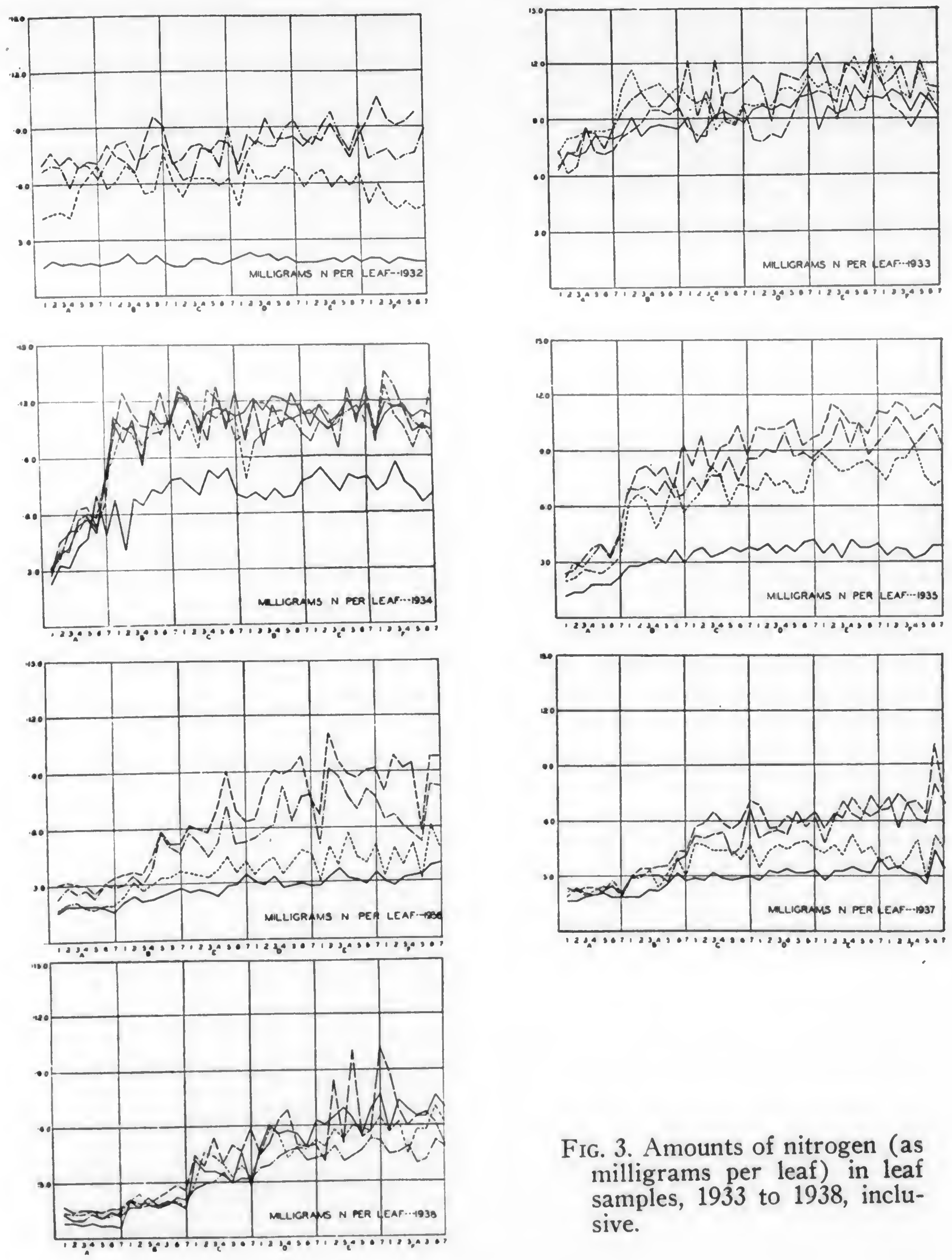


FIG. 3. Amounts of nitrogen (as milligrams per leaf) in leaf samples, 1933 to 1938, inclusive.

DISCUSSION

An examination of Fig. 1 shows that, starting with 1934, in certain sample series the leaf weights reflected the nitrogen applications; that is, the trees receiving no nitrogen (row A) had lower leaf weights than the other rows. During the subsequent years this relationship became more pronounced, so that by 1938 there was a gradual, although somewhat irregular increase in leaf weights from row A (no nitrogen) through row E (four units of nitrogen). It is apparent from an examination of this figure that the first samples taken in each year did not always show significant differences among the experimental trees as did the later samples. Further, it appears that the leaf weights increased rather regularly on all trees from the first to third samplings, and in most years the average leaf weight for the fourth sampling was less than at the time of the third sampling.

Comparing these data with the nitrogen analyses shown in Figs. 2 and 3, it is apparent that the nitrogen figures (expressed either as per cent or milligrams per leaf) also reflect the nitrogen applications in much the same way as did the leaf weights. Neither the air-dry leaf weights nor the leaf nitrogen figures show any appreciable degree of correlation with organic matter in the green manure applications. The green manure applications did, however, contain appreciable amounts of nitrogen so that from tree A-1 to A-7, for example, there was a slight, but regular increase in nitrogen applications.

Since the average air-dry leaf weights are relatively easy to secure, and the chemical analyses considerably more difficult, the question as to whether the leaf weights reflect the nitrogen applications as well as the nitrogen analyses naturally presented itself. Further, since it was obvious that there was considerable variation between the results secured on the different sampling dates, it was of considerable importance to know at what period of the growing season the greatest degree of correlation between nitrogen applications and the two "yardsticks" adopted was to be expected.

To answer these questions, correlation coefficients were calculated, first between nitrogen applications and the average air-dry leaf weights for each corresponding tree, and secondly, between nitrogen applications and leaf nitrogen analyses expressed as milligrams of the element per leaf. For each year the amounts of nitrogen applied up to that year were used to correlate with the leaf weights or nitrogen data, for example, the leaf weights and leaf nitrogen in 1937 were correlated with the amounts of nitrogen applied through 1936, and so on. The correlation coefficients thus calculated are presented in Table II.

TABLE II—Correlation Coefficients Between Grams of Nitrogen Applied and (A) Average Air-Dry Leaf Weights; (B) Milligrams of Nitrogen per Leaf on Various Sampling Dates

Year	First Sampling*	Second Sampling*	Third Sampling*	Fourth Sampling*	Fifth Sampling*
		A—Average Air-Dry Leaf Weights			
1933	0.608 ±0.066	0.210 ±0.099	0.190 ±0.100	0.443 ±0.084	
1934	0.566 ±0.071	0.580 ±0.069	0.566 ±0.071	0.607 ±0.066	0.671 ±0.057
1935	0.644 ±0.061	0.777 ±0.041	0.779 ±0.041	0.822 ±0.034	—
1936	0.291 ±0.095	0.575 ±0.070	0.854 ±0.028	0.716 ±0.051	—
1937	0.225 ±0.099	0.306 ±0.094	0.721 ±0.050	0.717 ±0.051	—
1938	0.885 ±0.022	0.885 ±0.022	0.702 ±0.053†	0.767 ±0.043‡	—
		B—Milligrams of Nitrogen Per Leaf			
1933	0.833 ±0.032	0.648 ±0.060	0.643 ±0.061	0.652 ±0.060	—
1934	0.645 ±0.061	0.638 ±0.062	0.595 ±0.067	0.666 ±0.058	0.676 ±0.056
1935	0.747 ±0.046	0.797 ±0.038	0.874 ±0.024	0.890 ±0.022	—
1936	0.807 ±0.036	0.832 ±0.032	0.871 ±0.025	0.826 ±0.033	—
1937	0.838 ±0.031	0.688 ±0.055	0.863 ±0.026	0.916 ±0.016	—
1938	0.939 ±0.012	0.930 ±0.014	0.827 ±0.033†	0.933 ±0.013‡	—

*See Table I for sampling dates.
†Terminal leaves, September 26.
‡Spur leaves, September 26.

From Table II it appears that there is considerable variation in the degree of correlation between air-dry leaf weights and nitrogen applications, particularly in the first sampling. This confirms the observations made previously from a casual examination of the data in Fig. 1. The data further indicate that in general the highest degree of correlation appears to result when samples are taken from late August to early October (third or fourth samplings). Excluding the results for the first year (1933), the correlation coefficients for the last two sampling periods in all years were highly significant, ranging from 0.566 to 0.854.

The correlation between leaf nitrogen and nitrogen applications is in general considerably higher than those mentioned in the preceding paragraph between leaf weights and nitrogen applications, and much more consistent, both from year to year and from month to month. The figures presented in the second half of Table II indicate that the nitrogen analyses of leaves taken in any of the four samplings gave a reasonably accurate reflection of the nitrogen applications, although there was a slightly higher degree of correlation late in the season.

Excluding the data for the first year (1933) which were inconclusive, it may be said that the degree of correlation between air-dry leaf weights and nitrogen applications in these trees was highly significant, provided the samples were taken during the late summer, from late August to early October. Thus it may be possible for a worker not equipped to make chemical analyses to secure through measurements of air-dry leaf weights a fairly accurate picture of the nitrogen nutrition of apple trees. The chemical analyses of the leaves, however, appears to furnish a more consistently reliable reflection of nitrogen fertilization.

Conclusions

A statistical study of 1050 apple leaf samples taken from 42 trees in the period between their sixth and eleventh years, fertilized with increasing amounts of nitrogen under closely controlled conditions gave the following results:

1. Nitrogen analyses of the leaves consistently reflected the fertilizer nitrogen applications, the coefficients of correlation ranging from 0.595 ± 0.067 to 0.939 ± 0.012.
2. Air-dry leaf weights exhibited an irregular but usually high degree of correlation with fertilizer nitrogen applications, the coefficients of correlation ranging from 0.190 ± 0.100 to 0.885 ± 0.022.
3. The results obtained during the first year of this study were irregular, but during the subsequent years the air-dry leaf weights correlated best with fertilizer nitrogen applications late in the growing season, from late August to early October, at which time r varied from 0.566 to 0.854.
4. Leaf nitrogen analyses showed a rather consistent degree of correlation with fertilizer nitrogen applications throughout the growing season, with a slightly higher degree of correlation late in the summer. The coefficients of correlation varied from 0.595 to 0.939,

and were higher than those found between air-dry leaf weights and nitrogen fertilization.

5. There was no apparent relation between green manure applications and either air-dry leaf weights or leaf nitrogen analyses.

Reprinted from THE JOURNAL OF NUTRITION
Vol. 26, No. 3, September, 1943

THE INFLUENCE OF THE THIAMINE INTAKE OF THE PIG ON THE THIAMINE CONTENT OF PORK WITH OBSERVATIONS ON THE RIBOFLAVIN CONTENT OF PORK[1]

R. C. MILLER, J. W. PENCE, R. A. DUTCHER, P. T. ZIEGLER
AND M. A. McCARTY

Department of Agricultural and Biological Chemistry, and Department of Animal Husbandry, Pennsylvania State College

(Received for publication March 13, 1943)

It is well-established that pork is an outstanding source of thiamine. A survey of the literature reveals, however, that there is considerable variation in the thiamine values which have been reported for pork. While some of these differences might be attributed to methods of determination, and to normal or expected variation, it is logical to suppose that certain physiological factors might also be responsible, at least in part, for some of the diversity among reported thiamine values for pork.

Among such factors are thiamine saturation of tissues (Schultz, Light and Frey, '38; Schultz, Light, Cracas and Atkin, '39), the relationship of thiamine to the glycogen cycle in muscle (Pyke, '40), and the thiamine sparing action of fats (Ellis and Madsen, '42).

It is a generally accepted fact that increased intake of carbohydrate increases the thiamine requirement; Waisman and Elvehjem ('41) have commented on this fact in connection with swine which are fed rations characteristically rich in carbohydrate. It is possible that the characteristically high thiamine content of pork is the result of demands on the muscle tissue for thiamine to aid in the metabolism of carbohydrate, including its conversion to fat.

---

[1] Authorized for publication on March 11, 1943, as paper no. 1171 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Part of the expenses of this investigation were defrayed by a fellowship grant from Swift and Company, Chicago, Illinois.

A further possibility is that the thiamine content of pork might be directly influenced by the thiamine intake of the pig. Some data bearing on this point have been presented by Pyke ('40) who reported thiamine values for the longissimus dorsi and psoas (back and loin) muscles from groups of two, five and four pigs fed, respectively, on swill, commercial meal (190 I. U. thiamine per 100 gm.), and the same commercial meal supplemented with crystalline thiamine to raise its thiamine content to 850 I. U. per 100 gm. No assay values are reported for the swill, but it is stated that it almost certainly contained less vitamin B_1 than the cereal meal. The amount of thiamine in the psoas muscle was shown to be consistently greater than that present in the longissimus dorsi. The thiamine values for these muscles were very similar for the pigs fed the plain meal and for those fed the meal supplemented with thiamine, but the values were considerably higher, in each instance, than those for the two swill-fed pigs. Pyke concludes that a normal pig ration supplies all the vitamin B_1 that the pig is capable of storing, and that the reduction in muscle thiamine in the swill-fed pigs is due to a deficiency of thiamine in the diet.

The present investigation was undertaken to obtain further data dealing with the relationship between the thiamine intake of the pig and the thiamine content of the pork. Although the study was primarily concerned with thiamine, riboflavin values were also determined owing to the paucity of riboflavin values reported in the literature.

EXPERIMENTAL

The feeding experiment was conducted during the period July to November, 1942. Twenty-seven pigs were assigned to three lots of nine pigs each, with the distribution between lots being made as nearly equal as was possible with respect to breed, sex and initial live weight (table 2). Throughout most of the feeding period the pigs were kept on the ground in half-acre outdoor feed lots which were devoid of vegetation. Suitable shelter against the sun and inclement weather was provided, and in the latter part of the experiment, in colder weather, the pigs were housed indoors.

The pigs were trough-fed in groups, twice daily. Data on feed consumption were recorded and no more feed was offered than could be readily consumed. A suitable amount of water was added to the feed at feeding time.

TABLE 1

Composition of rations.

	INGREDIENTS		
	Lot 1	Lot 2	Lot 3
	%	%	%
Yellow corn	57.0	49.0	53.5
Tankage (60% protein)	5.5	5.5	11.0
Soybean oil meal	10.0	10.0	—
Linseed oil meal	10.0	10.0	—
Dehydrated alfalfa leaf meal	5.0	5.0	10.0
Whole wheat	—	10.0	15.0
Peanut skins	10.0	10.0	—
Corn gluten meal	—	—	10.0
Dried brewers' yeast	2.0	—	—
Salt	0.48	0.48	0.48
Manganese	0.02	0.02	0.02
	100.00	100.00	100.00
	ANALYSIS		
Protein (%)	19.95	19.35	19.78
Thiamine (μg. per lb.)	5761	3447	1315
Riboflavin (μg. per lb.)	1801	1669	2468

The composition of the rations is given in table 1. The protein content of the rations is considered adequate for pigs weighing 40 to 50 pounds, but it is higher than that required for pigs weighing from 75 to 200 pounds. Rations 1, 2 and 3, fed to lots 1, 2 and 3, respectively, were designed primarily to furnish three levels of thiamine, the respective amounts being 5761, 3447 and 1315 μg. of thiamine per pound of feed. Extraordinary sources of thiamine were red peanut skins, used in rations 1 and 2, and brewers' yeast, used in ration 1. Some difficulty was experienced in obtaining brewers' yeast which contained sufficient thiamine to provide the desired amount in ration 1. Consequently, the required additions of pure thiamine (in solution) were mixed with this ration just prior to feeding.

Three pigs, one from each lot, were slaughtered on the 100th day of the feeding experiment. Thereafter, usually at weekly intervals, one pig from each lot was slaughtered. Thus the length of the feeding period varied from 99 to 140 days, respectively, for the first and last pigs slaughtered from each lot. After slaughter the carcasses were chilled for at least 24 hours at 37°F. before samples of the pork were taken for analysis. Samples were taken from the ham end of the loin, the center of the loin, the shoulder and the liver. Adhering fat was removed from the samples which were prepared for analysis by grinding in a food chopper. Thus, all analyses represent lean tissue.

Whenever possible, samples for the determination of dry matter, crude protein and thiamine were weighed immediately after the sample had been ground and thoroughly mixed. Otherwise samples were frozen immediately in the freezing compartment of an electric refrigerator and kept in the frozen state until they were analyzed. All of the riboflavin determinations were made on samples which had been kept in the frozen state.

Thiamine was determined by the thiochrome method, using the procedure recommended for cereal products (Hennessy, '42), and riboflavin was determined by the fluorometric method of Conner and Straub ('41). These methods were modified only in the manner of preparation of the extracts; a weighed amount of the previously ground fresh or frozen sample was mixed in a Waring blender with a suitable volume of extractant, from which mixture aliquots were pipetted into volumetric flasks.

TABLE 2

Weights, feed consumption and economy of gains of pigs with different levels of thiamine intake.

LOT NO.	THIAMINE CONTENT OF FEED	PIG NO.	BREED	SEX	AGE AT START OF EXPERIMENT	NUMBER OF DAYS ON FEED	INITIAL LIVE WEIGHT	FINAL LIVE WEIGHT	TOTAL GAIN IN LIVE WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY FEED	FEED REQUIRED PER 100 LBS. GAIN IN LIVE WEIGHT
	µg/lbs.				*days*		*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	5761	988	Chester White	Barrow	88	99	55	209	154	1.56	4.7	301
		990	Poland China	Barrow	74	105	49	207	158	1.50	4.8	319
		996	Chester White	Barrow	88	111	56	205	149	1.34	5.0	372
		995	Duroc Jersey	Gilt	74	112	44	200	156	1.39	5.0	361
		65	Duroc Jersey	Gilt	74	119	50	205	155	1.30	5.2	395
		70	Berkshire	Gilt	77	127	44	205	161	1.27	5.3	329
		74	Chester White	Gilt	88	133	34	205	171	1.29	5.4	422
		916	Poland China	Barrow	74	140	49	197	148	1.06	5.5	522
				Average	80	118	48	204	157	1.34	5.1	378
2	3447	992	Chester White	Gilt	88	99	61	226	165	1.67	4.9	297
		991	Chester White	Barrow	88	105	56	211	155	1.48	5.0	340
		998	Chester White	Barrow	88	111	49	200	151	1.36	5.2	382
		997	Duroc Jersey	Gilt	74	112	50	210	160	1.43	5.2	365
		66	Duroc Jersey	Barrow	74	119	47	220	173	1.45	5.2	278
		71	Poland China	Barrow	74	127	41	222	175	1.38	5.4	390
		75	Berkshire	Gilt	82	133	35	202	167	1.26	5.5	435
		917	Berkshire	Barrow	102	140	31	167	136	0.91	5.4	560
				Average	84	118	46	207	160	1.37	5.2	381
3	1315	994	Chester White	Barrow	88	99	52	212	160	1.62	5.0	308
		993	Duroc Jersey	Gilt	74	105	50	200	150	1.43	5.1	354
		999	Duroc Jersey	Barrow	74	111	45	200	155	1.40	5.2	375
		1000	Duroc Jersey	Gilt	74	112	47	218	171	1.53	5.3	345
		68	Chester White	Gilt	88	119	47	210	163	1.37	5.3	260
		73	Berkshire	Barrow	82	127	45	185	140	1.10	5.4	492
		915	Chester White	Gilt	88	133	49	198	149	1.12	5.5	494
		918	Poland China	Barrow	74	140	40	210	170	1.21	5.7	466
				Average	80	118	47	204	157	1.35	5.3	387

EXPERIMENTAL RESULTS AND DISCUSSION

Data pertaining to the body gains and feed consumption of the pigs are given in table 2. It is shown that, on the average, in spite of individual differences, the three lots of pigs were quite similar with respect to growth and feed requirements. It is apparent that the ration for lot 3, which furnished the least amount of thiamine was fully adequate to fulfill the pigs' requirements for this factor. In this case the average daily feed intake of 5.3 lbs. supplied 7 mg. of thiamine which is in excess of the suggested daily requirement of 1 to 3 mg. per 100 lbs. of body weight. The results show, further, that there was no apparent advantage derived from the additional thiamine provided in the rations of lots 1 and 2 so far as growth and feed utilization are concerned.

TABLE 3

Average proximate analyses of pork.

LOT NO.		1	2	3
NUMBER OF PIGS		8	8	8
THIAMINE CONTENT OF FEED (μG./LB.)		5761	3447	1315
SOURCE OF SAMPLE		%	%	%
Shoulder	Dry matter	27.0	28.5	28.2
	Protein in fresh pork	19.2	19.2	19.5
Center loin	Dry matter	27.8	27.6	29.2
	Protein in fresh pork	21.7	21.8	21.8
Ham end of loin	Dry matter	26.9	28.0	28.4
	Protein in fresh pork	21.4	21.8	21.2
Liver	Dry matter	30.5	31.0	30.5
	Protein in fresh liver	23.1	22.5	22.2

The average proximate analyses of the pork are given in table 3. Again, in spite of individual differences, there is general uniformity of the results for the pigs of the three lots.

Data for the thiamine content of the pork are given in table 4. Thiamine values for the muscle tissues directly reflect

TABLE 4

The thiamine content of pork as influenced by the thiamine content of the feed (thiamine expressed as μg./gm. of fresh tissue, dry matter and protein).

PIG NO.	SHOULDER			CENTER LOIN			HAM END OF LOIN			LIVER		
	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein
Lot 1 — 5761 μg. of thiamine per pound of feed												
988	16.0	51.7	87.7	20.1	61.8	99.0	22.3	80.2	106.8	5.9	19.3	24.2
990	13.1	44.2	65.7	18.3	69.9	81.3	17.8	65.5	80.9	2.7	9.5	12.2
996	19.4	76.6	95.2	25.1	86.1	110.9	26.3	93.5	120.9	7.3	24.5	31.8
995	17.9	63.8	96.1	25.3	87.8	112.3	24.4	93.9	120.5	6.2	20.7	26.7
65	14.7	59.2	80.3	23.3	86.5	109.6	22.6	87.8	110.2	6.2	19.9	27.5
70	17.2	69.5	85.4	23.8	89.5	106.1	21.8	81.7	97.7	3.6	11.9	16.2
74	19.2	71.2	102.1	22.8	79.6	105.4	25.8	90.9	123.6	5.6	17.3	24.5
916	21.1	82.5	109.2	27.8	106.9	125.3	29.9	117.0	132.5	5.2	16.6	21.4
Average	17.3	64.8	90.2	23.1	83.5	106.2	23.9	88.8	111.6	5.3	17.5	23.1
Standard error	0.9	4.5	4.8	1.0	4.8	4.4	1.3	5.2	5.6	0.5	1.7	4.8
Lot 2 — 3447 μg. of thiamine per pound of feed												
992	13.1	41.8	69.4	19.9	69.6	90.5	18.6	63.2	85.3	4.8	15.5	20.1
991	16.8	57.7	86.7	23.4	80.9	106.7	24.2	86.8	108.8	4.8	15.7	20.4
998	15.5	51.1	78.5	18.3	65.3	82.2	19.6	67.1	85.4	4.4	15.3	21.9
997	16.9	57.5	87.5	20.7	77.8	92.0	19.4	70.3	93.5	5.1	15.9	21.5
66	12.6	49.6	67.0	20.1	77.1	94.6	19.3	76.0	91.3	5.5	18.0	23.0
71	14.0	46.2	75.4	18.8	65.8	90.9	19.2	64.2	89.8	4.2	14.2	18.9
75	13.8	52.7	69.0	19.0	69.0	84.0	19.9	72.9	88.2	3.9	11.8	18.1
917	17.8	68.3	92.5	20.1	76.7	93.8	20.5	75.8	95.3	3.0	9.3	14.2
Average	15.1	53.1	78.3	20.0	72.8	91.8	20.1	72.0	92.2	4.5	14.5	19.8
Standard error	0.7	2.9	2.9	0.6	2.1	2.6	0.6	2.7	2.7	0.3	1.0	1.0
Lot 3 — 1315 μg. of thiamine per pound of feed												
994	6.5	20.1	35.6	9.2	28.7	44.3	10.4	37.3	52.0	4.2	13.9	18.6
993	5.9	23.5	31.0	7.3	24.5	32.7	11.1	38.5	52.1	3.8	11.9	15.1
999	10.1	37.4	49.0	9.6	30.9	43.1	10.0	33.1	48.3	3.6	10.7	16.6
1000	7.7	25.1	41.3	9.2	31.5	45.3	8.8	28.6	44.3	3.5	11.5	15.2
68	7.1	28.0	33.9	10.5	40.4	45.7	9.5	36.4	42.2	2.5	8.2	12.1
73	9.7	35.2	46.6	10.4	36.7	45.8	12.1	43.4	52.6	1.9	6.4	8.2
915	7.5	26.4	39.1	9.7	35.9	44.0	9.5	33.8	43.3	2.4	8.2	10.5
918	8.9	30.6	47.3	10.3	33.9	49.9	11.2	41.3	55.1	4.5	15.5	24.1
Average	7.9	28.3	40.5	9.5	32.8	43.9	10.3	36.6	48.7	3.3	10.8	15.1
Standard error	0.5	2.1	3.1	0.4	1.8	1.8	0.4	1.7	1.7	0.3	1.1	1.8

the thiamine intakes of the pigs, and the data show that much higher amounts of thiamine were present in the muscle tissues of the pigs of lots 1 and 2 than was the case for similar tissues of the pigs of lot 3. The thiamine content of the pork from the pigs of lot 2, which received 3447 µg. of thiamine per pound of feed, is approximately twice that of the pigs of lot 3, which received 1315 µg. of thiamine per pound of feed. In the case of lot 1, which received 5761 µg. of thiamine per pound of feed, the average thiamine values for the pork are higher, in all instances, than those for the pork from the next lower thiamine lot (no. 2), but, although the trend of the values seems to be established, the differences are not statistically significant in all cases. The above relationships are true whether or not the comparisons are made on the basis of the thiamine content of the fresh pork, dry matter, or total protein. Although the thiamine values for the pork from lot 1 are higher than those for lot 2, the differences are not commensurate with the differences in the thiamine intake of these two lots. A more direct relationship exists in the case of lots 2 and 3 where an increase of 2.6 times in thiamine intake resulted in approximately doubling the thiamine content of the pork.

It is interesting to note that there seems to be a preferential attraction of pork muscle for thiamine as compared to liver. Furthermore, liver seems to be much less responsive to the effect of added dietary thiamine. It will be noted, in comparing lots 2 and 3, that the increase in thiamine content of liver, as the result of thiamine feeding, was of the order of 40 to 70%, as contrasted with about 100% for muscle. Pork muscle is unique in its ability to absorb and retain thiamine and appears to be the principal repository of this vitamin.

Our findings with respect to the increased thiamine content of pork muscle as the result of feeding are somewhat similar to those reported by Pyke ('40), but they differ in several respects the first of which is the magnitude of the thiamine values for pork from pigs with more or less comparable intakes of thiamine.

The limited data of Pyke show a range of from 12 to 14.55 µg. of thiamine per gram of loin on feeds which ranged from 2586 to 11,567 µg. of thiamine per pound of feed. Our thiamine values are somewhat higher, ranging from 18.3 to 27.8 µg. per gram of loin from pigs which received 3447 and 5761 µg. of thiamine per pound of feed. In spite of the difference in the magnitude of the thiamine values, our observations and those of Pyke show that different muscle tissues from the same pig may contain different amounts of thiamine. In our studies the loin values exceeded those of the shoulder by 20 to 25%. No data are available showing whether there may be a variation in the thiamine content of different parts of the same muscle.

Pyke's results show little, if any, increase in the thiamine values for pork when the level of thiamine feeding was increased from 2586 to 11,567 µg. per pound of feed, although the values at these levels were approximately double those for swill-fed hogs. Our values for pigs fed 3447 µg. of thiamine per pound of feed are approximately 30% higher than those reported by Pyke at the highest level of feeding (11,567 µg. per pound of feed), and in each instance our values show some increase as a result of a higher level of thiamine intake.

There does not appear to be any satisfactory explanation for the above points of difference. Feed consumption might be a factor influencing thiamine storage. Pyke does not report the feed intakes, but in our case it was practically the same for the three lots of pigs. Our results do not indicate any marked influence of breed on the thiamine content of the pork. However, Pyke's data indicate that breed might be a factor, and it is possible that the Wessex Saddleback pig might differ in this respect from the breeds used in our investigation. We feel, however, that the species rather than the breed, in the case of swine, characterizes the great ability of the pig to accumulate thiamine in its muscle tissue.

Our results, which show that the thiamine content of pork may be influenced by the thiamine intake of the pig, and that different pork cuts may vary in thiamine content, are of particular interest in helping to explain some of the wide variations in thiamine values for pork which have been reported in the literature. Our work does not fully delineate the limits within which the thiamine content of pork may vary, but some indication of the upper limit is indicated by the results obtained by feeding 5761 μg. of thiamine per pound of feed to lot 1, and by further data given in table 5.

TABLE 5

Thiamine and riboflavin values for pork from pigs receiving 50 mg. of additional thiamine hydrochloride daily for a period of 22 days

PIG. NO.		69[1]	72[2]	69	72
SOURCE OF SAMPLE	BASIS	THIAMINE		RIBOFLAVIN	
		μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*
Shoulder	Fresh	15.5	14.1	3.5	3.7
	Dry matter	59.8	50.1	13.5	13.1
	Protein	79.2	71.6	17.9	18.7
Center loin	Fresh	22.2	18.6	1.8	2.4
	Dry matter	89.2	66.2	7.4	8.4
	Protein	101.5	86.3	8.4	10.9
Ham end of loin	Fresh	21.8	18.1	2.9	3.3
	Dry matter	85.7	66.3	11.3	12.0
	Protein	102.3	84.7	13.4	15.3
Liver	Fresh	5.6	5.8	51.7	46.9
	Dry matter	19.0	17.0	175.3	137.5
	Protein	26.0	23.8	239.8	192.4

[1] Pig no. 69 received feed containing 5761 μg. thiamine per pound.

[2] Pig no. 72 received feed containing 1315 μg. thiamine per pound.

One pig from the original lot 2 was affected with piles and was removed from the experiment. Its mates in lots 1 and 3, nos. 69 and 72, were continued on the experiment and received the same feeds as the other members of lots 1 and 3, with the addition, during the last 22 days of the experiment, of a daily supplement of 50 mg. of pure thiamine. The results, as given in table 5, show that there was no accumulation of this additional thiamine in the muscle tissue of the pig (no. 69) on the high thiamine (5761 μg. per pound) feed; however, a very appreciable storage of thiamine did occur in the case of pig no. 72, which had received only 1315 μg. of thiamine per pound of feed. The thiamine values for the pork of this pig are of the same order as those for the pork of the pigs of lots 1 and 2, and indicate that thiamine may be very rapidly accumulated in pork muscle tissue under conditions of high thiamine intake.

Schultz, Light, Cracas and Atkin ('39) have pointed out that storage of thiamine in the accepted sense, does not take place in the body of the rat. We feel that probably this is also true in the case of the pig. This viewpoint may involve a very strict differentiation between storage and accumulation. Since our limited data indicate that thiamine may be accumulated rapidly in pork muscle, under conditions of high thiamine intake, it is felt that its disappearance from the tissue may be equally rapid. Thus, thiamine accumulation in the tissue, even to the point of saturation, may not constitute true storage.

The data presented in this paper do not give a complete picture with respect to the average values and the lower limit of the thiamine values which may apply to pork in general and which may be determined by the thiamine intake of the average hog. Limited data are available for the thiamine content of hog feeds. It may be assumed, however, that many hog rations, especially those in which corn predominates heavily, are not rich in thiamine. On the other hand, hogs fed peanuts, peanut by-products, certain oil meals, yeast and other thiamine-rich feed ingredients ingest considerable amounts of thiamine. With the lack of any specific data to prove the point, we feel that 1315 μg. of thiamine per pound of feed, the amount present in the ration of lot 3, represents a thiamine intake for hogs which is probably somewhat higher than the average. Thus, the thiamine values for the pork of lot 3 are probably at least not lower than the average.

Average riboflavin values for pork from lots 1, 2 and 3 are given in table 6. No attempt was made to control the riboflavin content of the feeds. Consequently, the variations in this vitamin in the feeds, were not comparable to those of thiamine. It will be noted that the riboflavin content of pork muscle is much lower than that of thiamine while pork liver is rich in riboflavin but relatively low in thiamine.

TABLE 6

The riboflavin content of pork.

LOT NO.		1		2		3	
NUMBER OF PIGS		8		8		8	
RIBOFLAVIN CONTENT OF FEED (μG./LB.)		1801		1669		2468	
SOURCE OF SAMPLE	BASIS	AVERAGE RIBOFLAVIN CONTENT OF PORK (EXPRESSED AS μG./GM. OF FRESH TISSUE. DRY MATTER AND PROTEIN)					
		μg./gm.	*Standard error*	*μg./gm.*	*Standard error*	*μg./gm.*	*Standard error*
Shoulder	Fresh	3.5	0.1	3.3	0.1	3.2	0.1
	Dry matter	13.0	0.7	11.5	0.5	11.5	0.9
	Protein	18.2	0.6	17.0	0.7	16.4	0.7
Center loin	Fresh	2.2	0.1	2.3	0.1	2.2	0.1
	Dry matter	7.9	0.4	8.1	0.4	7.5	0.4
	Protein	10.1	0.4	10.3	0.6	10.1	0.3
Ham end of loin	Fresh	3.2	0.2	3.0	0.2	3.0	0.2
	Dry matter	11.8	0.7	10.6	0.5	10.7	0.6
	Protein	14.9	1.0	13.6	0.6	14.3	0.7
Liver	Fresh	43.8	0.9	41.8	0.8	40.5	1.0
	Dry matter	143.7	3.0	134.9	2.3	132.7	2.7
	Protein	189.6	2.9	185.9	3.0	182.5	3.5

SUMMARY

Three lots of pigs were fed rations containing 5716, 3447 and 1318 μg. of thiamine per pound of feed, respectively. The average feed consumption was approximately the same for the three lots of pigs. Thus, the average daily thiamine intakes were approximately 29, 17 and 7 mg., respectively.

Results with respect to growth and feed consumption were similar for the three lots of pigs in spite of the differences in thiamine intake.

Thiamine values were determined on pork samples taken from the shoulder, the middle of the loin, the ham end of the loin and the liver. An increase of approximately 100% in the thiamine content of pork muscle resulted when the thiamine intake was increased from 1318 to 3447 μg. per pound of feed. A similar increase of the order of 15 to 20% resulted from an increase in the thiamine content of the feed from 3447 to 5761 μg. per pound.

The average thiamine values, on the fresh basis, for shoulder, center loin and ham end of loin, at the lower level of thiamine intake, were 7.9, 9.5 and 10.3 μg. per gram, respectively. At the high level of thiamine intake the corresponding values were 17.3, 23.1 and 23.9 μg. per gram.

The thiamine content of pork loin was found to be consistently higher than that of pork shoulder. Pork liver, as compared to muscle, was found to be relatively low in thiamine content. There was some increase in the thiamine content of the liver as the result of feeding more thiamine, but the main depository of thiamine in the pig is the muscle tissue.

Limited observations with but two pigs seem to indicate that pork muscle tissues have nearly reached the saturation point, so far as thiamine storage is concerned, when the thiamine content of the ration approximates 5800 μg. per pound. As the result of these preliminary observations it is concluded that thiamine accumulation in pork muscle is relatively rapid. These experiments are being continued.

Riboflavin values were determined for all of the pork samples. The riboflavin content of pork muscle is low as compared to this tissue's content of thiamine. Pork liver is relatively high in riboflavin content.

LITERATURE CITED

CONNER, R. T., AND G. J. STRAUB 1941 Combined determination of riboflavin and thiamin in food products. J. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 385.

ELLIS, N. R., AND L. L. MADSEN 1942 The thiamin requirements of pigs as related to the fat content of the diet. J. Animal Sci., vol. 1, p. 84.

HENNESSY, D. J. 1942 A standard thiochrome assay for the determination of thiamin in cereal products. The Cereal Chemists' Bull., vol. 2, p. 25.

PYKE, MAGNUS 1940 Vitamin B_1 in vertebrate muscle. Biochem. J., vol. 34, p. 1341.

SCHULTZ, A. S., R. F. LIGHT AND C. N. FREY 1938 Vitamin B_1 metabolism in man. Excretion of B_1 in urine and feces. Proc. Soc. Exp. Biol. and Med., vol. 38, p. 401.

SCHULTZ, A. S., R. F. LIGHT, L. J. CRACAS AND L. ATKIN 1939 The concentration of vitamin B_1 in the tissues of the rat. J. Nutrition, vol. 17, p. 143.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. The Burgess Publishing Co., Minneapolis, Minn., p. 85.

Reprinted from the July—October 1943 Issue of the RECORD OF CHEMICAL PROGRESS

VITAMINS

R. ADAMS DUTCHER

Some New Aspects of Vitamin A Chemistry

IN 1932 English workers postulated that vitamin A may be made to undergo partial decomposition when the blue solution obtained with antimony trichloride is mixed with water (Edisbury, Gillam, Heilbron and Morton, Biochem J., *26*, 1164 (1932)). These workers postulated that the new compound was probably cyclic in structure. It was also observed that this "spurious" or "cyclized" vitamin A could be made by treating vitamin A with HCl in alcohol.

Shantz, Cawley and Embree (J. Amer. Chem. Soc., *65*, 901 (1943)) have concluded that "cyclized vitamin A" is really formed by dehydration. For this reason they prefer to call the product "anhydro vitamin A." Using a vitamin A concentrate, these workers have formed anhydro vitamin A by dehydration with N/30 anhydrous alcoholic hydrogen chloride. By chromatographic methods it is possible to obtain the anhydro compound in the eluate. The concentrate thus obtained may be crystallized at $-60°$ C. if permitted to stand for about two days in low boiling petroleum ether. The crystals are described as being orange yellow in color, melting at 76 to 77°. Comparisons of absorption spectra of vitamin A and anhydro vitamin A and of the antimony trichloride reaction products of vitamin A and anhydro vitamin A indicate that the anhydro compound is a separate entity.

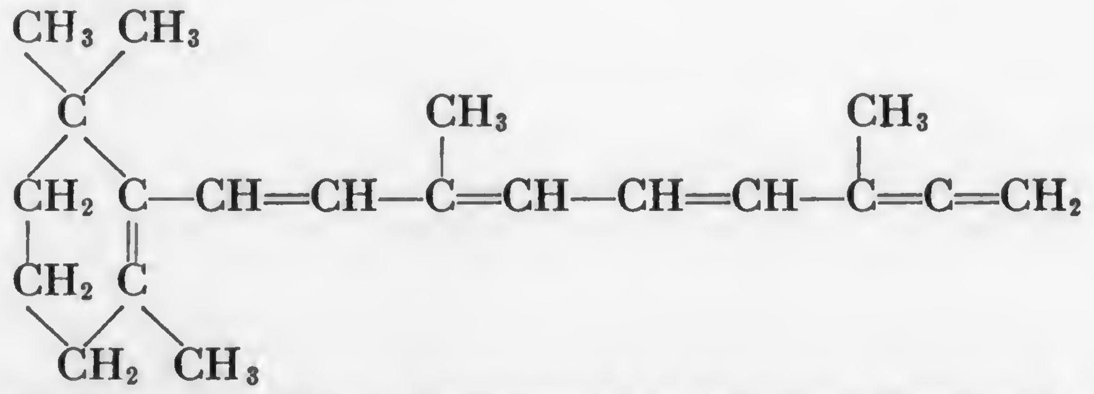
Polymerized orange and red compounds may be formed in the reaction mixture which can be separated from the anhydro compound by molecular distillation. A compound which is provisionally called "Isoanhydro vitamin A" is formed if vitamin A is permitted to remain in contact with the acid reagent for a prolonged period of time at room temperature. Carbon and hydrogen analyses support the belief that vitamin A ($C_{20}H_{30}O$) has been dehydrated to form anhydro vitamin A ($C_{20}H_{28}$). Absence of hydroxyl groups was shown by failure to obtain methane when anhydro vitamin A was treated with methylmagnesium iodide in isoamyl ether. Hydrogenation of anhydro vitamin A led to the conclusion that the molecule contains six unsaturated bonds.

Stability studies, as measured by absorption spectra, indicate that anhydro vitamin A is stable in oil solutions but that crystalline preparations and purified concentrates deteriorate quite rapidly. The dehydrated product was also shown to differ markedly from vitamin A when these compounds were subjected to analytical molecular distillation.

When refluxed for several hours with a lower alkyl alcohol, vitamin A esters break down to anhydro vitamin A, free fatty acids and "other products." Anhydro vitamin A prepared by this method is identical with that prepared by the acid-alcohol method, previously described.

When the biological potency of the dehydrated product was determined it was found that it contained 15,200 units of vitamin A per gram, while the product obtained by ester decomposition assayed 16,900 units per gram. These were thought to be reasonably free from traces of vitamin A.

Possible structures for anhydro vitamin A are discussed without arriving at definite conclusions. The authors are inclined to feel that existing optical absorption evidence points to the fact that the following "allene" structure is most likely to be correct, although methylene and dimethylene structures are not excluded.



Suggested "Allene" Structure for Anhydro Vitamin A

A practical example of the usefulness of the dehydration method is the separation of vitamins A_1 and A_2. These are ordinarily separated with considerable difficulty. However, their anhydro derivatives are separated quite readily by chromatography. In its highest purity, the crystalline hydrocarbon, anhydro vitamin A, has a value of 3650 for *E* (1%, 1 cm.) (371 mμ).

Embree and Shantz (J. Amer. Chem. Soc., *65*, 906 (1943)) have applied the dehydration procedure to the unsaponifiable portion of fish liver oils. In this paper they discuss the applications of chromatography to the separation of vitamins A_1 and A_2. Anhydro vitamin A_2 is adsorbed quite strongly on alumina, while anhydro vitamin A_1 is washed through the column with petroleum ether. Both possess the same ultraviolet absorption spectrum.

When the unsaponifiable fraction of shark liver oil was subjected to the dehydration procedure, it was found that a new substance could be identified by spectrographic methods. This new substance was similar to anhydro vitamin A but differed in the position of the three absorption bands. Furthermore, it appeared that the new compound contained one less double bond in conjugation. Since they were unable to detect the new substance before dehydration, it was reasoned that it must have been formed during the dehydration process from some "progenitor." This progenitor has been tentatively designated as "subvitamin A" and its dehydrated product as "anhydro subvitamin A."

The absorption spectrum of subvitamin A differs from that of vitamin A_1 although, with antimony trichloride the former gives a blue color comparable with that of vitamin A_1. The deportment of subvitamin A in the analytical molecular still was unlike that of vitamins A_1 and A_2.

While the antimony trichloride method indicated that one sample of subvitamin A (in oil) had an apparent potency of 3000 I.U./gm., biological tests on four rats indicated that the subvitamin A possessed no growth promoting power. If this is true, additional evidence is afforded to show that chemical methods should not be depended on to the exclusion of biological response methods. Embree and Shantz suggest that subvitamin A is possibly an oxygenated derivative of vitamin A_1 or A_2.

In 1937 Prichard and co-workers described a substance present in whale liver oil which possessed characteristic absorption spectra, contained no vitamin A and displayed no change in spectral characteristics when treated with alcoholic hydrogen chloride. In spite of these facts, the fraction possessed an activity of 17,900 I.U./gm. when fed to rats (Prichard, Wilkinson, Edisbury and Morton, Biochem. J., *31*, 258 (1937)).

Embree and Shantz (J. Amer. Chem. Soc., *65*, 910 (1943)) have applied their techniques to whale liver oil and have described the properties of this potent substance which they have named "kitol," from the Greek word *ketos* meaning whale.

Kitol was isolated from whale liver oil by dissolving the unsaponifiable fraction in ethyl formate, freezing out the sterols, dissolving the sterol-free material in corn oil and distilling at 150° C. in a cyclic molecular still. The kitol remained in the residue. After further saponification, the unsaponifiable fraction, containing kitol, was subjected to chromatographic fractionation on aluminum oxide. Following two additional adsorptions and elutions with alcohol and ether a pale yellow oil was obtained which soon set to a glassy solid.

Thermal decomposition studies yielded the interesting information that at temperatures of 175° C. or higher a substance was formed which gave a blue color with antimony trichloride and the color had an absorption maximum of 620 mμ, like vitamin A. In addition the decomposition product had an absorption band with a maximum at 328 mμ. It distributed itself between 83% alcohol and petroleum ether like vitamin A and its dehydrated product possessed spectral characteristics identical with anhydro vitamin A.

Biological assay of one sample of the decomposition product revealed a vitamin A potency of 42,000 I.U./gm. It was concluded that kitol is not a vitamin but a provitamin, since the kitol concentrate yielded negative results when tested by the official bioassay method. It was estimated that the decomposition of one molecule of kitol possibly yields one molecule of vitamin A.

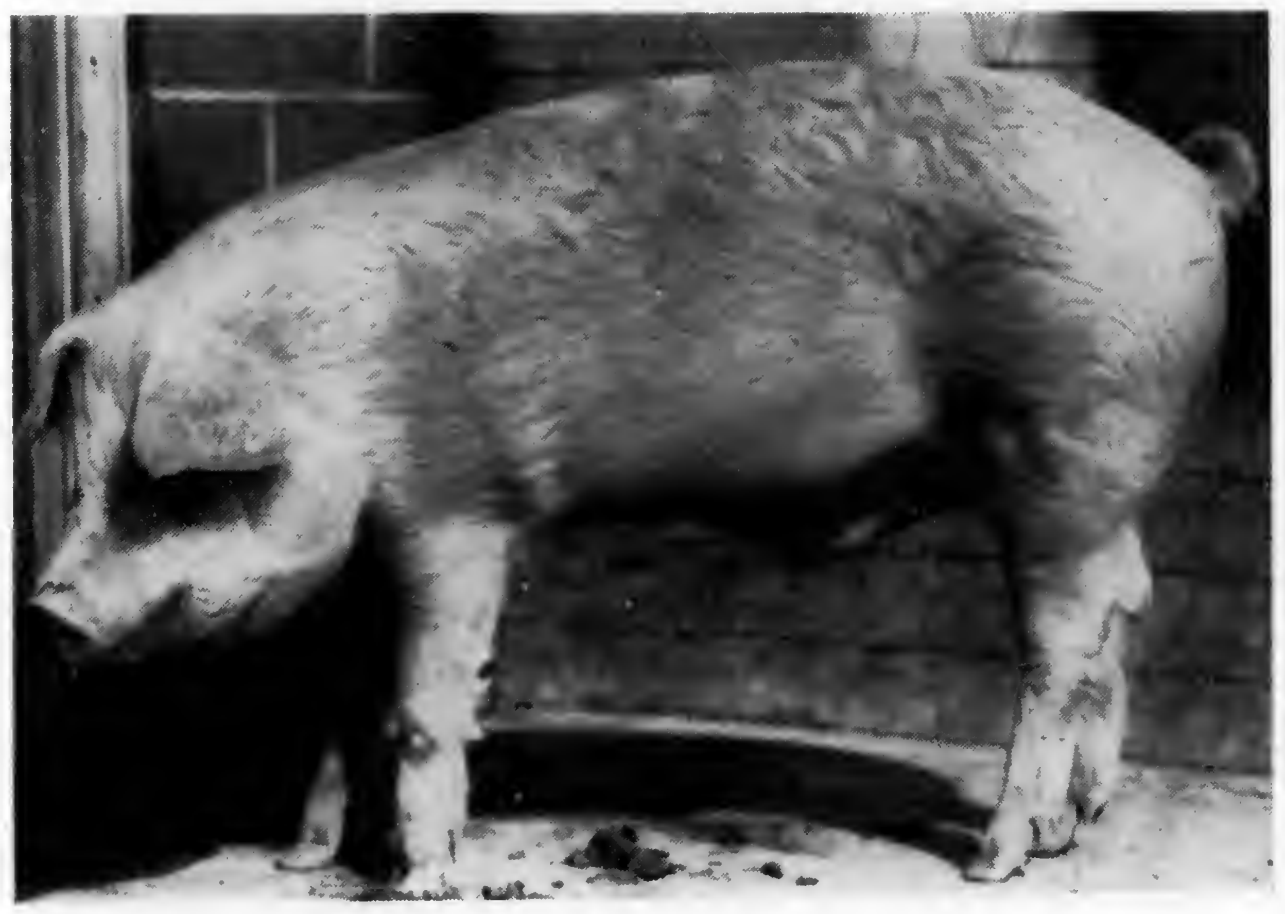
Kitol has a molecular weight of about 575, and it is a dihydric alcohol with the approximate formula $C_{40}H_{60}O_2$. It is thought that 8 double bonds are present in the molecule.

Kitol is found in small amounts in shark liver oil and in lamb liver oil. The authors found a product in pike liver oil which seemed to bear the same relationship to vitamin A_2 that kitol bears to vitamin A_1. As a result they postulate the existence of a kitol_2 in pike liver oil.

Since vitamin A is unstable to heat, the authors suggest that heating of kitol by ordinary methods would not be commercially satisfactory, if vitamin A were to be produced by thermal decomposition of kitol. The only feasible method, they contend, would be to use a molecular still where vitamin A can be removed as fast as it is formed.

BULLETIN 456 NOVEMBER 1943

Feed Mixtures for Young Pigs



The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

DIGEST

Dehydrated alfalfa meal was found slightly superior to sun-cured alfalfa meal for the young pig. No difference was observed in the nutritive value of these two feedstuffs for pigs weighing 75 pounds and over. The vitamin A content of the two rations may account for the differences observed with the younger pigs. Abnormalities observed in the individuals fed both rations were probably caused by deficiencies of a factor or factors other than vitamin A.

Rations in which white-cap corn replaced yellow corn were decidedly inferior for the normal development of the young pig.

Cod liver oil did not improve the ration containing white-cap corn in so far as growth was concerned. However, pigs fed cod liver oil did not develop specific symptoms of vitamin A deficiency which were shown by pigs on the white-cap corn ration.

Rations high in soybean oilmeal proved to be superior to rations low in soybean oilmeal and all other rations used, with respect to growth, hair and skin conditions, and gait and general development, for pigs of the initial weight of 37 to 44 pounds.

For pigs weighing 37 to 44 pounds at the beginning of the experiment, the replacement with soybean oilmeal of part of the low protein tankage in the ration resulted in improved growth and condition of the pigs as judged by the hair, skin, gait and general development.

Feed Mixtures for Young Pigs*

T. B. Keith, R. C. Miller, M. A. McCarty and W. T. S. Thorp †

MIXTURES OF FEEDS commonly fed to swine in the absence of good forage may be inadequate for normal growth and development of the young pig. Some of the factors affecting the growth and death losses of pigs from date of birth to the time they reach market weight have been discussed in a previous publication.[1]

In other work dealing with the protein and the manganese requirements of the young pig, certain abnormalities were observed with pigs fed a ration composed of white-cap corn 58.5 parts, whole alfalfa meal 12 parts, 34 per cent protein tankage 25 parts, expeller soybean oilmeal 4 parts, salt ½ part, and manganese 0.25 pound per ton. Out of a total of five pigs, one died during the fourth week of feeding, the other four being normal with respect to gains. One of these pigs, however, had a curved spine at the end of 18 weeks of feeding, fig. 1. This individual weighed 41 pounds at the beginning of the experiment and made an average daily gain of 0.57 pounds up to the time of the appearance of the curvature. This abnormality appeared to be similar to the curvature of the spine of the pigs fed a vitamin A deficient mixture at the Kansas Experiment Station.[2]

The experiments reported here were planned for the purpose of studying the best means of overcoming these and related deficiencies in an otherwise practical ration. The feedstuffs studied were dehydrated alfalfa meal, ground sun-cured alfalfa hay, expeller process soybean oilmeal, white-cap corn, yellow corn, and the supplementary value of cod liver oil in a white-cap corn mixture for young growing pigs, table 1.

Feedstuffs.—The yellow corn used in these tests was purchased from a nearby feed dealer. All of the kernels were deep yellow in color. It was graded as No. 2 and contained 9 per cent crude protein.

The white-cap yellow corn was grown on the College farm. It was of grade No. 2 and contained 9.6 per cent crude protein. The color of the kernels varied from a white to a yellow with a white cap.

---

* Authorized for publication, October 7, 1943.

† Assistant Professor of Animal Husbandry, Associate Professor of Agricultural and Biological Chemistry, Associate Professor of Animal Husbandry, and Associate Professor of Animal Pathology Research, respectively.

[1] "Nutritive Requirements of Young Pigs", Pennsylvania State Agricultural Experiment Station Bulletin No. 449, 1943.

[2] "The Importance of Vitamin A and Vitamin C in the Ration of Swine," Kansas State Agricultural Experiment Station Technical Bulletin No. 23, 1928.

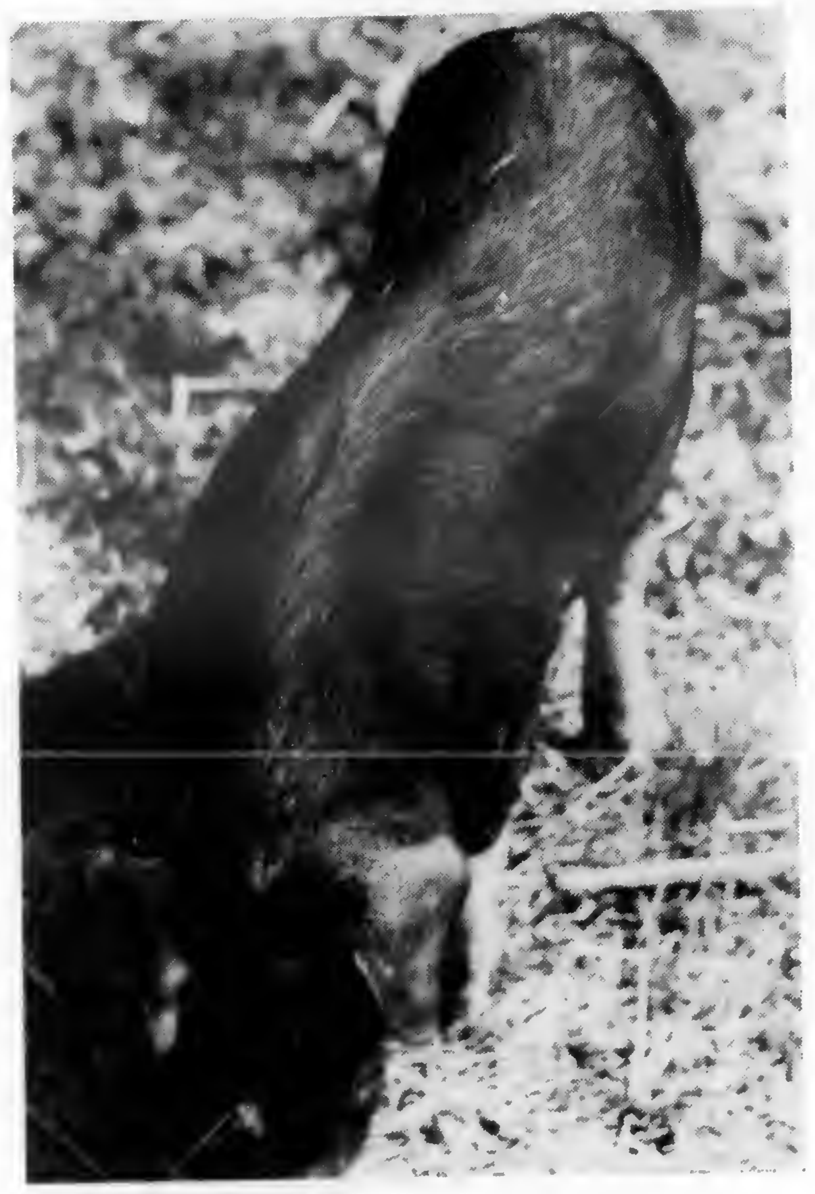


Fig. 1.—This pig had a curved spine after being fed on a concrete feeding floor for 18 weeks on a ration composed of white-cap corn, ground alfalfa hay, 34 per cent protein tankage, expeller soybean oilmeal, salt, and manganese.

The sun-cured alfalfa meal was made from hay of first and second cuttings and contained both stems and leaves. It was grown on the College farm and nearby farms and was ground in a hammer mill with a three-thirty-seconds-inch screen. It contained 20.4 per cent crude protein and varied in color from pale to deep green.

The dehydrated alfalfa meal was made from the stems and leaves and had a deep green color. It contained 21.3 per cent crude protein.

The tankage, a low protein (34 per cent) product, was obtained from a local meat packing plant. It was made from slaughter house waste consisting of material derived from sheep, cattle, and hogs in about equal quantities.

Table 1.—Composition of six different rations fed to young growing pigs.

	RATIONS					
COMPONENTS	1	2	3	4	5	6
	parts	*parts*	*parts*	*parts*	*parts*	*parts*
Yellow corn	45.5	45.5	47.5	49.5		
White-cap corn					47.5	47.5
Tankage (34 per cent protein)	22	22	25	13	25	25
Soybean oilmeal	22	22	15	25	15	15
Sun-cured alfalfa hay	10		12	12	12	12
Dehydrated alfalfa hay		10				
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Cod liver oil						2 *

* Cod liver oil was added to the daily feed allowance.

SUN-CURED ALFALFA MEAL vs. DEHYDRATED ALFALFA MEAL

Since alfalfa meal was the main source of vitamin A in the ration in which a deficiency apparently occurred, and since alfalfa contains varying quantities of vitamin A, a comparison was made of the relative values of sun-cured alfalfa meal and dehydrated alfalfa meal in identical mixtures, rations 1 and 2, table 1. The pigs were fed in matched pairs; one member of each pair was fed the sun-cured alfalfa and the other was fed dehydrated alfalfa. Two experiments were conducted, 10 pairs of pigs being fed during the summer months and 8 pairs during the winter months. The pigs fed during the summer months were placed on feed in July, and those fed during the winter months were placed on feed in December.

With both the summer and the winter fed groups, there was no marked difference between the average daily gain of the pigs fed the sun-cured or dehydrated alfalfa mixtures from initial weights of 30 to 50 pounds, up to 75 pounds, or the initial weights up to the final weights, which ranged from 154 to 173 pounds, tables 2 and 3.

Table 2.—Gains, weights, feed consumption, and economy of gains of 18 pairs of pigs between average initial weights of 30 to 50 pounds and a final weight of 75 pounds. One member of each pair received sun-cured alfalfa meal; the other member received dehydrated alfalfa meal.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
				Beginning July 1940				
Sun-cured alfalfa	10	74	72	30	68.9	.62	1.85	310
Dehydrated alfalfa	10	75	72	31	70.3	.61	1.72	284
				Beginning December 1940				
Sun-cured alfalfa	8	89	74	49	54.3	.46	1.67	368
Dehydrated alfalfa	8	95	75	50	50.8	.49	1.66	343

The pigs fed the dehydrated alfalfa meal mixtures required 26 pounds or 8.4 per cent less feed for each 100 pounds gain during the summer months, and 25 pounds or 6.8 per cent less feed for each 100 pounds gain during the winter months, than the pigs fed the sun-cured alfalfa rations, up to a weight of 75 pounds.

The differences in the feed requirements of the same pigs, shown in table 2, fed the sun-cured and the dehydrated alfalfa mixtures between the initial weights and average final weights of 154 and 173 pounds for either the summer or the winter group, are not large enough to be significant, table 3.

These data indicate that the younger and lighter weight pig requires more of the nutrients that are supplied by the dehydrated alfalfa meal mixture, and not supplied by the sun-cured alfalfa

Table 3.—Gains, weights, feed consumption, and economy of gains of the same 18 pairs of pigs as in table 2, between average initial weights of 30 to 50 pounds and average final weights of 154 and 173 pounds. One member of each pair received sun-cured alfalfa meal; the other member received dehydrated alfalfa meal.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
			Beginning July 1940					
Sun-cured alfalfa	10	74	155	30	151	.82	3.06	376
Dehydrated alfalfa	10	75	154	31	153	.80	3.02	377
			Beginning December 1940					
Sun-cured alfalfa	8	89	173	49	125	.93	3.50	378
Dehydrated alfalfa	8	95	172	50	144	.91	3.54	393

meal mixture, than the older, heavier pig. They indicate further that any advantage, as judged by the feed requirement, gained by the pigs fed dehydrated alfalfa up to weights of approximately 75 pounds was not sustained in the later period of growth and development. This may indicate some advantage for the sun-cured hay during this latter period.

However, neither the sun-cured nor dehydrated alfalfa meal mixtures are considered to be nutritionally adequate, since some pigs fed each of these mixtures showed stiffness or incoordination in the legs, and since instances of growth failure were observed in pigs fed both rations.

WHITE-CAP CORN vs. YELLOW CORN

Since the pig shown in fig. 1 with the curved spine was fed white-cap corn, a comparison was made of the gains and general performance of pigs fed white-cap corn and pigs fed yellow corn, rations 3 and 5, table 1. The pigs were fed as matched pairs, one member of each pair being fed a mixture with yellow corn, the other member being fed the same mixture with yellow corn replaced by white-cap corn. A summary of the results is presented in table 4.

The pigs fed the yellow corn ration made 0.08 pound or 14 per cent greater average daily gain and required 9 pounds, or 3 per cent less feed for 100 pounds gain between the initial weight of 48 to 51 pounds and a final weight of 75 pounds.

Table 4.—Gains, weights, feed consumption, and economy of gains of 5 pairs of pigs between the average initial weight of 50 pounds and the average final weight of 75 pounds. One member of each pair received yellow corn; the other member received white-cap corn.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
Yellow corn	5	89	75	51	36	.67	2.15	321
White-cap corn	5	82	71	48	39	.59	1.95	330

Two of the pigs fed the white-cap corn were partially blind. All of the pigs on this ration were growthy in appearance. They were angular, hair coats were rough, their skins were dry, and their backs were sharp with little or no meat on their bodies, figs. 2 and 3.

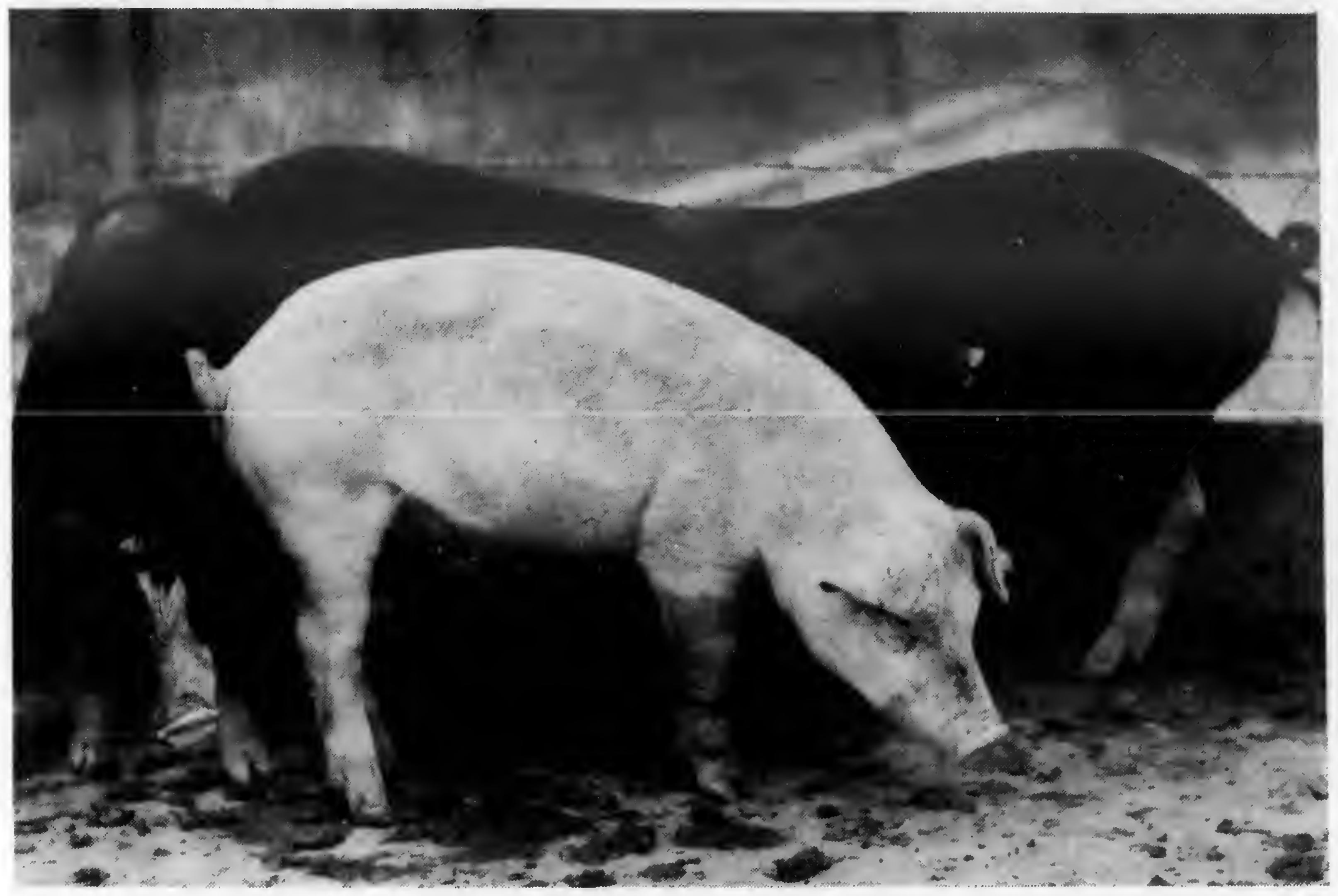


Fig. 2.—Four pigs fed the white-cap corn mixture. (Ration 5) These pigs were angular and had an uneven flesh covering. Two of these pigs were partially blind; one died from pneumonia.

Fig. 3.—Poland China barrow fed white-cap corn mixture (Ration 5). Note the coarse appearance of the hair, the dry skin, the angularity of conformation, and the general unthriftiness. This animal was partially blind.

The gains and feed requirements were similar for those individuals which finished the test on both rations, between the weight of 75 pounds and the average final weight of 184 pounds. One individual fed the yellow corn ration and the paired-mate fed the white-cap corn ration were fed 100 grams of dried beef liver daily after failing to gain normally during the first 10 weeks of feeding. Both individuals resumed normal growth and finished the feeding trial in excellent condition. Two pigs fed the white-cap corn ration had pneumonia; one died and on autopsy its lungs were found to be congested.

COD LIVER OIL AS A SUPPLEMENT TO WHITE-CAP CORN

Four pairs of pigs were selected for a study of the value of adding 2 per cent of cod liver oil to the white-cap corn ration. One member of each pair was fed the white-cap corn ration and the other member was fed the white-cap corn mixture with 2 per cent cod liver oil, rations 5 and 6, table 1. Between an average initial weight of 49 pounds and a final weight of 73 pounds, the pigs fed the white-cap corn mixture with 2 per cent cod liver oil made 0.04 pound or 9 per cent more daily gain, and required 16 pounds, or 4 per cent less feed for each 100 pounds of gain, than the pigs fed the white-cap corn mixture without the cod liver oil, table 5.

Table 5.—Gains, weights, feed consumption, and economy of gains of 4 pairs of pigs between the average initial weight of 49 pounds and the average final weight of 73 pounds. One member of each pair received white-cap corn and the other member received white-cap corn with 2 per cent of cod liver oil.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
White-cap corn	4	78	72	50	51	.43	1.71	396
White-cap corn and cod liver oil	4	79	73	49	51	.47	1.79	380

The data relating to the gains and to the feed requirements of the same pigs reported in table 5, from an average initial weight of 49 pounds to an average final weight of 164 pounds, are reported in table 6. The pigs fed the white-cap corn without cod liver oil made 0.04 pound or 5 per cent more daily gain with 22 pounds, or 5 per cent less feed than the pigs fed the white-cap corn mixture plus 2 per cent of cod liver oil. While indicating a slight trend in favor of the cod liver oil ration at the lighter body weights, these data show no advantage for this ration over the entire feeding period.

In general appearance there were no distinct differences in the individuals fed the white-cap corn mixture and the individuals fed the white-cap corn mixture with 2 per cent cod liver oil. The pigs fed both rations were angular in appearance, sharp over the back, lacked a uniform meat covering, and the hair coat was rough and dry. One individual fed the white-cap corn without cod liver oil became dizzy. Another pig fed the same mixture was sensitive in the joints of the legs and vertebrae.

Table 6.—Gains, weights, feed consumption, and economy of gains of the same 4 pairs of pigs as in table 5, between the average initial weight of 49 pounds and the average final weight of 164 pounds. One member of each pair received white-cap corn, and the other member received white-cap corn with 2 per cent of cod liver oil.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
White-cap corn	4	78	164	50	133	.86	3.44	400
White-cap corn and cod liver oil	4	79	158	49	133	.82	3.46	422

The eyes of the four pigs fed the white-cap corn mixture without cod liver oil and of two of the pigs fed the white-cap corn mixture with cod liver oil, were examined with an opthalmoscope. The individuals fed white-cap corn had changes in the optic nerve, described as papillary edema and cloudiness of the disc. No distinct changes were observed in the pigs fed the cod liver oil.

HIGH SOYBEAN OILMEAL vs. HIGH TANKAGE MIXTURES

A comparison was made of a high soybean oilmeal and low tankage ration with a high tankage and low soybean oilmeal ration for growing pigs, rations 3 and 4, table 1. These pigs were fed in matched pairs; one member of each pair was fed the high soybean oilmeal ration and the other member was fed the high tankage ration. Two experiments were conducted; 5 pairs were fed during the summer months and 6 pairs were fed during the winter months. The data are presented in table 7.

The pigs fed the high soybean oilmeal mixture during the summer months for the growing period between the initial weights

Table 7.—Gains, weights, feed consumption, and economy of gain of 11 pairs of pigs between average initial weights of 37 to 44 pounds, and the average final weight of 75 pounds. One member of each pair received a high tankage low soybean oilmeal ration; the other member received a low tankage high soybean oilmeal ration.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
			Beginning July 1940					
High tankage	5	91	74	44	45	.70	2.05	293
High soybean oilmeal	5	85	75	44	41	.80	1.96	239
			Beginning December 1940					
High tankage	6	93	75	37	75	.50	1.72	339
High soybean oilmeal	6	88	73	38	69	.52	1.60	306

and the average final weights of 75 pounds made 0.10 pound, or 14 per cent more daily gain, and required 54 pounds, or 18 per cent less feed for each 100 pounds gain than the pigs fed the high tankage ration.

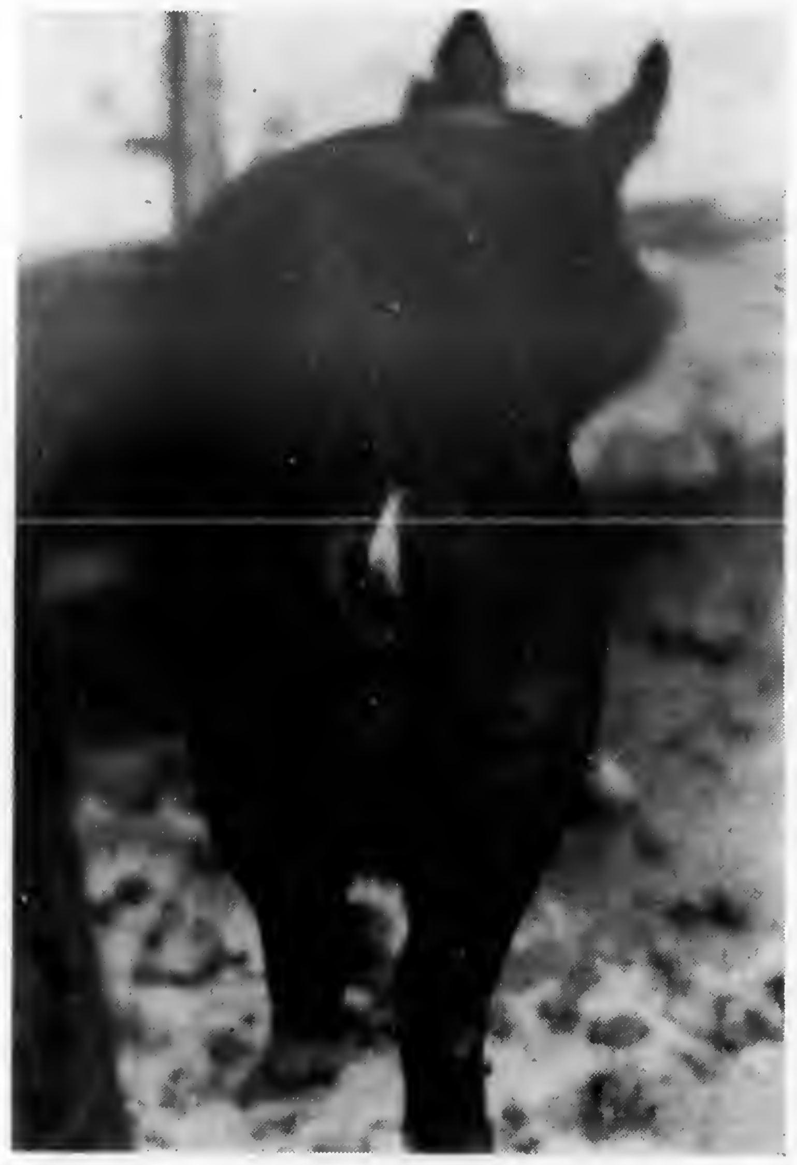


Fig. 4.—Berkshire sow fed yellow corn in a high soybean oilmeal and low tankage ration (Ration 4). Note smoothness, width of back, and depth of fleshing.

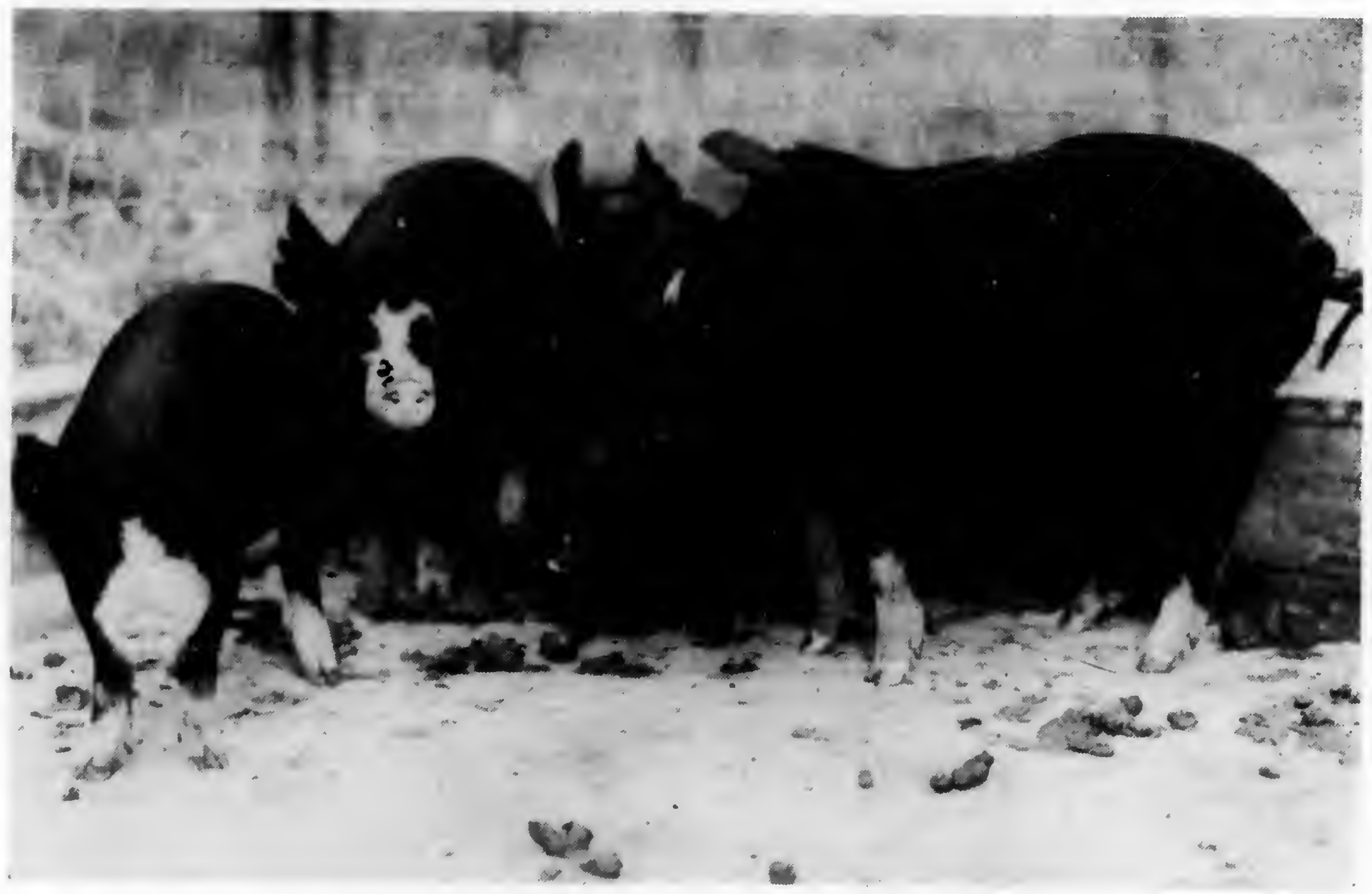


Fig. 5.—Group of pigs fed yellow corn in a high soybean oilmeal ration (Ration 4). Note depth of fleshing and smoothness.

Among the 5 pairs, 4 pigs fed the high soybean oilmeal ration made more rapid gains and required less feed for each 100 pounds of gain than the paired mates fed the high tankage ration.

The pigs fed the high soybean oilmeal ration during the winter months for the period between the initial weights and the average final weights of 75 pounds, made 0.02 pound more average daily gain and required 33 pounds, or 10 per cent less feed for each 100 pounds of gain than the pigs fed the high tankage ration. Of the 6 pairs, every pig fed the high soybean oilmeal ration required less feed for each 100 pounds of gain than the paired mates fed the high tankage ration.

The pigs fed the high soybean oilmeal ration were more uniformly fleshed, were not so narrow over the top line, and their hair coats were not so coarse and dry in appearance, figs. 4 and 5.

The pigs were continued on these mixtures to final weights of approximately 139 and 150 pounds, table 8. The pigs which were fed the high soybean oilmeal ration made more rapid gains and required less feed for each 100 pounds of gain than the pigs fed the high tankage ration. These differences were not so great as the differences found in the comparison of the pigs fed the high soybean oilmeal and the high tankage ration for the period between the initial weight and 75 pounds.

Table 8.—Gains, weights, feed consumption, and economy of gain of the same 11 pairs of pigs as in table 7, between average initial weights of 37 and 44 pounds and average final weights of 145 and 191 pounds. One member of each pair received a ration high in tankage; the other member received a ration high in soybean oilmeal.

RATION	NO. PIGS	AGE	FINAL WEIGHT	INITIAL WEIGHT	DAYS ON FEED	AV. DAILY GAIN	AV. DAILY RATION	FEED PER 100 LB. GAIN
		days	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
			Beginning July 1940					
High tankage	5	91	186	44	144	.99	4.08	412
High soybean oilmeal	5	85	196	44	144	1.08	4.09	378
			Beginning December 1940					
High tankage	6	93	136	37	133	.74	2.67	361
High soybean oilmeal	6	88	155	38	133	.87	2.71	311

DISCUSSION

Six experiments with a total of 80 pigs were conducted to determine the value of sun-cured alfalfa meal, compared with dehydrated alfalfa meal; yellow corn compared with white-cap corn; the addition of cod liver oil to the white-cap corn mixture for the young growing pig; and a high soybean oilmeal low tankage mixture, compared with a high tankage low soybean oilmeal mixture. A total of 18 pigs were fed the sun-cured alfalfa ration, 18 were fed the dehydrated alfalfa ration, 11 were fed the high tankage ration, and 11 were fed the high soybean oilmeal ration, 13 were fed the white-cap corn ration, and 5 were fed the yellow corn ration.

The feed requirement of the pigs fed dehydrated alfalfa meal was less than that of the pigs fed sun-cured alfalfa meal for the period between the time of weaning and the time when the pigs weighed 75 pounds. No significant differences were observed in gains, feed requirements, and general appearance of the pigs fed the dehydrated alfalfa meal mixture and the sun-cured alfalfa meal mixture when the whole feeding period from weaning to final weight is considered.

When white-cap corn replaced yellow corn in the same mixture, the resulting growth and development indicated that the nutritive requirements of the pig were not fulfilled. Two of the pigs fed the white-cap corn were partially blind. One died from pneumonia. All of the pigs fed the white-cap corn ration appeared emaciated and unhealthy. The addition of cod liver oil did not improve the white-cap corn ration with respect to growth. However, pigs which did not receive cod liver oil showed pathological changes in the eyes which are attributed to a deficiency of vitamin A.

The young pigs fed a mixture containing 25 per cent soybean oilmeal and 13 per cent tankage required 10 to 18 per cent less feed for each 100 pounds of gain than the pigs fed the mixture containing 15 per cent soybean oilmeal and 25 per cent tankage for the period between the time of weaning and the time the pigs weighed 75 pounds. The nutritive requirements of the older and heavier pigs appeared to be satisfied with the low soybean oilmeal mixture.

A summary of some of the observations made on individual pigs fed the various rations is given in table 9.

Table 9.—Summary: Mortalities and Abnormalities.

RATION	TOTAL NUMBER OF PIGS USED	NUMBER OF PIGS WHICH DIED	NUMBER OF STIFF PIGS	REMARKS
Sun-cured alfalfa	18	2	3	Two had a dermatitis on ears, tails, and legs. All had indications of a dermatitis.
Dehydrated alfalfa	18	none	2	Three had a dermatitis on ears, tails, and legs. All had indications of a dermatitis.
High tankage	11	none	none	All had indications of a dermatitis.
High soybean oilmeal	11	none	none	Excellent skin condition.
White-cap corn	5	1	2	All had slight dermatitis, were emaciated, and had sharp backs. Two developed pneumonia. Two appeared stiff.
Yellow-corn	5	none	none	All had slight dermatitis.
White-cap corn	4	none	3	All had a slight dermatitis, several were partially blind, and all had sharp backs. Three appeared stiff and dizzy.
White-cap corn and cod liver oil	4	none	2	All had a slight dermatitis and sharp backs. Two appeared stiff.

Made in United States of America

Reprinted from The Journal of Physical Chemistry
Vol. 47, No. 9, December, 1943

pH MEASUREMENTS ON THIXOTROPIC GEL SYSTEMS USING THE GLASS ELECTRODE

R. C. VOGEL[1] AND M. W. LISSE

Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pennsylvania

Received September 3, 1943

Thixotropy is the reversible isothermal gel–sol transformation which may be brought about by stirring, shaking, or similar procedures. A thorough historical treatment of thixotropy is not necessary here, in view of the splendid review article written by H. Freundlich (3).

Within recent years the glass electrode and apparatus to be used in connection with it have been extensively developed. The glass electrode is the only instrument which is suitable for use in measuring the pH of materials in the gel state; accordingly, it is now possible to study the pH changes which may accompany the first gelation of a gel-forming material, the thixotropic liquefaction, and the subsequent re-gelation of the sol thus produced.

The literature reveals a number of studies of pH changes during the processes preceding the first gelation of a gel-forming material (1, 5, 7, 8, 9, 10), but none appears to have been made during the complete first gelation of a gel-forming material, the thixotropic liquefaction, and the re-gelation of the sol produced by the thixotropic liquefaction. We shall call the process up to and including the first gelation "primary gelation" and the gelation of any sol originally a gel but liquefied by shaking "secondary gelation."

THEORETICAL CONSIDERATIONS

The scaffolding hypothesis (4, 6, 11) has been one of many which have been advanced to describe the structure of thixotropic gels. According to it the gel consists of a network of particles aggregated in the form of chains and cross chains, the particles being in actual contact. This structure is pictured as a scaffolding extending throughout the gel and providing complete molecular binding from one side of the container to the other (4). There is some experimental evidence supporting this hypothesis (4, 6, 11).

It is a well-known fact that gels, sols, and suspensions of particles approaching colloidal dimensions have very large surfaces on which adsorption could occur. If we consider the scaffolding hypothesis, might one not theorize that when particles link together there will be a decrease in surface? It is also a well-known fact that the hydrogen ion is often very actively adsorbed, and thus one might expect that a change in surface would bring about a change in pH of the dispersion medium, provided it is not buffered too much. If during primary gelation, thixotropic liquefaction, and secondary gelation changes in structure produce changes in surface available for adsorption, pH changes as well as changes in ionic activity of other ions might possibly be expected. The change in pH might be sufficiently large to be measurable. The fact that other investigators have observed pH changes during the early processes of primary gelations of a number of gels (1, 5, 7, 8, 10) might also lead one to expect pH changes during the later processes of primary gelation, the thixotropic liquefaction, and the secondary gelation; however, the pH changes noted during the initial processes of primary gelations might have been due to the establishment of equilibrium conditions after the mixing of the gel components and not in any way connected with the formation of the gel's structure.

EXPERIMENTAL PROCEDURE

The systems which were studied were thorium molybdate, bentonite, and ferric hydroxide. The gelation time of each system was determined using the inverted-tube method. It was realized that this method was quite arbitrary, but it has been used many times in investigations of thixotropy and is convenient for obtaining relative data. Broughton and Squires (2) discussed the inverted-tube method and concluded that it was capable of giving consistent results.

It was necessary to determine gelation times in vials of the same size as those in which the gel was to be placed during the pH determinations (inside height 2.8 cm., inside diameter 1.7 cm.), since the time of re-gelation of a thixotropic gel-forming material is dependent upon the amount of glass surface in contact with it and thus upon the dimensions of the vessel. Glass rods were made of the same size and shape as the glass and calomel electrodes, except in cases when a bridge made direct contact with the gel in place of the calomel electrode. Then glass rods were made of the same size and shape as the glass electrode and the end of the bridge dipping into the gel. The false glass electrode and the false calomel electrode or bridge, depending upon which was used during the pH determinations, were placed in rubber stoppers so that they could be held rigidly in the gel by placing the rubber stoppers in the gelation vials during the experiments used to determine the gelation times.

In all experiments the temperatures of the room and of the solutions were kept at 25°C. ± 1°.

RESULTS

Thorium molybdate system

The thorium molybdate gel-forming system was prepared by thoroughly mixing 2 ml. of a potassium molybdate solution (12.000 g. of K_2MoO_4 per liter), 5 ml. of thorium nitrate solution (55.481 g. of $Th(NO_3)_4 \cdot 4H_2O$ per liter), and 0.5 ml. of distilled water. When this mixture was allowed to remain undisturbed, a gel showing thixotropy was formed. The average primary gelation time of the gel was found to be 24 min. with a maximum variation of 3 min., and the average secondary gelation time 25 min. with a maximum variation of 3 min.

In preliminary attempts to measure the pH changes during the primary gelation, thixotropic liquefaction, and secondary gelation, the saturated calomel and glass electrodes were dipped directly into the gel-forming thorium molybdate. It was found that the gel-forming material would diffuse into the calomel electrode and solidify there, thus interfering with its operation.

---

[1] Present address: Department of Chemistry, Harvard University, Cambridge, Massachusetts.

It was obvious that some way must be found to avoid direct contact between the calomel electrode and the thorium molybdate gel-forming system. To accomplish this the glass electrode was placed in the gel-forming material and the calomel electrode in a separate vessel containing a saturated potassium chloride solution. The gel-forming material and the saturated potassium chloride solution were connected by a bridge. An agar bridge containing a saturated potassium chloride solution did not give satisfactory results, but a bridge containing thorium molybdate gel, prepared in the same manner as the gel-forming material, did solve the problem.

In pH determinations of the type using the glass and saturated calomel electrodes, there is always a liquid-junction potential between a saturated potassium chloride solution and the sample whose pH is desired. This liquid-junction potential is kept to a minimum, because the transference numbers of the potassium and the chloride ions are about the same and because the concentration of these ions is much larger than the concentration of the ions of the system in contact with the saturated potassium chloride solution. For this reason the diffusion of the potassium and chloride ions chiefly controls the liquid-junction potential. In the experiments with the thorium molybdate gel bridge, this liquid-junction potential occurred between the thorium molybdate in the bridge and the saturated potassium chloride solution. In the use of the glass electrode we are required to correct for its asymmetry potential by standardization with a standard buffer. As nearly as possible the liquid-junction potentials in the standardization and in the actual pH determinations should be about the same and consist of one junction potential between the saturated potassium chloride solution and the sample whose pH is being determined. If in the standardization the gel-forming thorium molybdate was merely replaced with the standard buffer, this would not have been the case, as there would have been junction potentials between the saturated potassium chloride solution and the thorium molybdate gel in the bridge and between the same material in the bridge and the standard buffer. For this reason, in the standardization the glass and calomel electrodes were dipped directly into the standard buffer, thus giving as nearly as possible liquid-junction potentials similar to those existing in the actual pH determinations.

After the pH meter had been standardized, the gel components were mixed, placed in the bridges, and allowed to solidify there. Then a fresh sample of the gel-forming material was made, 2 ml. of the mixture was pipetted into a gelation vial, and the pH readings were taken at specific times. After the material had been a gel for 6 min., the vial with its gel was removed from the bridge and glass electrode. The gel was liquefied by shaking, and the first pH readings made 25 sec. after liquefaction (removing, shaking, replacing of the liquefied gel, and taking of the first pH readings required about 1 min.). The readings were then continued during the secondary gelation. After each experiment with this system had been completed, the pH meter was checked with the standard buffer to make certain that it had been giving correct readings. The data given in table 1 are representative of four experiments performed on the thorium molybdate system using this method. The maximum variation in each group of four pH readings taken at a specific time after each experiment was started is 0.02 pH.

TABLE 1

pH changes in thixotropic gel systems

TIME	pH VALUES			
	Thorium molybdate system	Ferric hydroxide system	Bentonite system	
minutes				
1	2.57	4.50	4.87	Primary gelation
2	2.58	4.48	4.87*	
3	2.59	4.47	4.87	
4	2.61	4.46	4.87	
5	2.61	4.45	4.87	
6	2.62	4.44		
7	2.62	4.44		
8	2.62	4.43		
9	2.62	4.42		
10	2.62	4.42	4.87	
15	2.62	4.41	4.87	
20	2.62	4.40*	4.87	
25	2.62*	4.40	4.87	
30	2.62	4.40	4.87	
				Thixotropic liquefaction of all gels
31	2.62	4.40	4.87	Secondary gelation
32	2.62	4.40	4.87	
33	2.62		4.87	
34	2.62		4.87	
35	2.62	4.40	4.87	
40	2.62	4.40†	4.87	
45	2.62	4.40		
50	2.62	4.40	4.87†	
55	2.62†			
60	2.62			

* pH at approximate primary gelation time.

† pH at approximate secondary gelation time.

Ferric hydroxide system

The ferric hydroxide gel-forming system was prepared by mixing 10 ml. of 5 per cent "Dialyzed Iron" (Merck) with 5.3 ml. of distilled water and then adding to this 1 ml. of 0.493 N sodium hydroxide. This mixture was shaken vigorously, and when it was set aside a gel showing thixotropy was formed. The average primary gelation time was found to be 20 min. and the average secondary gelation time 10 min., both with a maximum variation of 4 min.

When the pH readings during the gelations were taken by placing the electrodes directly in the gel-forming mixture without any precautions, the initial readings of each experiment differed widely and non-reproducible pH changes upon thixotropic liquefaction were obtained. These difficulties could not be corrected by using bridges in the manner described under the thorium molybdate system.

Two things had to be done in order to obtain initial pH readings which were identical within the limits of error (±0.02 pH). If after each gelation experiment the glass and calomel electrodes were placed in concentrated hydrochloric acid for 5 min. and then washed off with distilled water, the initial pH readings when the electrodes were placed in the gel-forming material were the same. It was thought that the concentrated hydrochloric acid dissolved the material which had been strongly adsorbed from previous experiments on the glass electrode and thus interfered with its operation. It was also discovered that if the electrodes had been in standard buffer immediately preceding the gelation experiments erratic initial readings were obtained. This source of trouble was eliminated by allowing the electrodes to remain in a ferric hydroxide gel-forming system for an hour after they had been in the standard buffer and before they were treated with concentrated hydrochloric acid.

In preliminary experiments the procedure used during the thixotropic liquefaction giving inconsistent results was the following: After the last pH reading during the primary gelation, the gel in its vial was removed from the electrodes and shaken. The electrodes were washed off with distilled water and the excess water removed with lens paper. The electrodes were then replaced in the liquefied gel and the pH readings were taken during the secondary gelation. However, when the electrodes were removed from the gel and replaced in contact with the liquefied gel without any treatment, the variations obtained in pH when the gel was shaken disappeared.

The final experimental method used for the ferric hydroxide system was the following: Before each set of gelation experiments the glass and calomel electrodes were placed in concentrated hydrochloric acid for 5 min. and then washed off with distilled water. Using a standard buffer, the Beckman pH Meter was set to give correct readings. The electrodes which were used in the standardization were placed in the ferric hydroxide system for 1 hr., treated with concentrated hydrochloric acid for 5 min., and washed off with distilled water. They were then placed in 3 ml. of the freshly prepared gel-forming mixture, and pH readings were taken at intervals until the material had become a gel and constant pH readings were obtained. The gel and its vial were removed from the electrodes, shaken until the gel liquefied, and replaced in contact with the electrodes. The first reading during the secondary gelation was taken 25 sec. after the gel was liquefied (the removing of the gel, shaking, replacing of the liquefied gel, and taking of the first reading required about 1 min.). The pH readings were continued for about 10 min. after the system had again become a gel. After the experiment was complete, the electrodes were removed from the gel and washed off with distilled water, and the pH meter was again checked with the standard buffer to show that correct readings had been obtained. The complete process was repeated in each set of gelation experiments. The data given in table 1 are representative of four experiments performed on the ferric hydroxide system using the above method. The maximum variation in each group of four pH readings taken at a specific time after each experiment was started was 0.02 pH.

Bentonite system

The bentonite system was prepared from a bentonite suspension made by dispersing 20 g. of bentonite (Volclay from the American Colloid Company, Chicago, Illinois) in 1000 ml. of distilled water. This dispersion was allowed to settle for not less than 6 days nor more than 3 weeks. It was then decanted from the settled material before use. To 900 ml. of this decanted suspension was added 11 ml. of 0.197 *N* hydrochloric acid. The unacidified bentonite suspension had a pH of about 7.80; thus it was desirable to use an acid bentonite suspension to prepare the systems to be studied, since this avoided a possible error due to absorption of carbon dioxide from the air. The gelation times of a number of systems using various ratios of the suspension, 2.5 *N* potassium chloride, and distilled water were determined in order to find a ratio of reagents producing a system with desirable gelation times. A system having an average primary gelation time of 2 min. with a maximum variation of 2 min. and an average secondary gelation time of 20 min. with a maximum variation of 5 min. was made by diluting 200 ml. of the acidified bentonite suspension with 32.5 ml. of distilled water; then to 11.64 ml. of this suspension 0.37 ml. of 2.5 *N* potassium chloride solution was added drop by drop with shaking. The diluted acidified bentonite suspension used above contained 0.1233 g. of solid material per 10 ml.

The pH meter was set to give correct readings by using a standard buffer, and the electrodes were thoroughly washed with distilled water. Immediately after the gel-forming mixture, prepared as outlined above, was made, 4 ml. of it was pipetted into a gelation vial. The glass and calomel electrodes were placed directly in the mixture and the pH readings were taken at specific times. After the material had been a gel for approximately 28 min., it was removed from the electrodes and liquefied by shaking. During the thixotropic liquefaction the electrodes were not treated in any way. They were replaced and the first reading during the secondary gelation was taken 25 sec. after the gel was liquefied (the removing of the gel, shaking, replacing of the liquefied gel, and taking of the first reading required about 1 min.). The pH readings were continued until the material had undergone secondary gelation. The electrodes were not washed off with distilled water nor the excess gel material removed in any mechanical way before a new sample was introduced. Contrary to the procedure used in investigating the other systems, the pH meter was not checked with the standard buffer to make sure that it had been giving correct readings until all the gelation experiments had been completed; it seemed that a treatment similar to that used in the case of ferric hydroxide systems, which was thought to remove the standard buffer adsorbed on the glass electrode, was not sufficient to give good results for the bentonite system.

The data given in table 1 are representative of three experiments performed on the bentonite system using the above method. The maximum variation in each group of three pH readings taken at a specific time after each experiment was started is 0.03 pH.

DISCUSSION OF RESULTS

During the primary gelation the pH of a thorium molybdate system increased from 2.57 to 2.62, that of ferric hydroxide decreased from 4.50 to 4.40, and that of bentonite remained constant. During the thixotropic liquefactions and secondary gelations no changes in pH of any of the three systems were observed, using the glass electrode.

Prakash and Dhar (8), using a thorium molybdate system also, obtained increases in pH (from 2.16 to 2.39) with a quinhydrone electrode during the preliminary processes of primary gelation while the system still remained a sol. Their system was prepared by mixing 10 ml. of a thorium nitrate solution (48.1400 g. per liter) and 2 ml. of a potassium molybdate solution (12.0000 g. per liter). In the preparation of the authors' system more potassium molybdate was used, so it seems reasonable that their system would be more alkaline than that of Prakash and Dhar (the final pH of the authors' system was 2.62, as compared with 2.39). However, Prakash and Dhar continued pH readings for only 80 min., whereas the primary gelation time of their gel was 5 hr. and 30 min. Their last readings had been constant for 10 min. when they were discontinued.

The data here reported, as well as those of Prakash and Dhar, disagree with those of Prasad and Desai (9) who, with a quinhydrone electrode, also measured the pH changes of a thorium molybdate system during the early stages of primary gelation. They found the pH constant at 2.96. Their system was prepared by mixing 5 ml. of a thorium nitrate solution (60 g. per liter), 1 ml. of potassium molybdate solution (100 g. per liter), and 4 ml. of water.

In the case of the ferric hydroxide system Prakash and Dhar (8) found an increase in pH during the early stages of primary gelation from 2.91 to 3.26. They prepared their system by mixing the following solutions: 6 ml. of $M/2$ ferric chloride, 3.75 ml. of 2.84 N sodium acetate, 1.5 ml. of 4 N ammonium sulfate, and 0.45 ml. of 4.26 N ammonium hydroxide. The volume was made up to 30 ml. by adding water. Perhaps the difference in the direction of pH change during the primary gelations might be due to the fact that there were different ions in the two ferric hydroxide systems which were undergoing adsorption.

SUMMARY

1. During the primary gelations ferric hydroxide and thorium molybdate gel-forming systems showed pH changes, but a bentonite system showed no pH change.

2. During the thixotropic liquefaction there is no pH change within the limits of experimental error in the cases of a thorium molybdate, a ferric hydroxide, and a bentonite gel.

3. During the secondary gelation there is no change in pH within the limits of experimental error in the cases of a thorium molybdate, a ferric hydroxide, and a bentonite system.

REFERENCES

(1) Batchelor, H. W.: J. Phys. Chem. **42**, 575 (1938).
(2) Broughton, G., and Squires, L.: J. Phys. Chem. **40**, 1041 (1936).
(3) Freundlich, H.: *Thixotropy*, Actualités scientifiques et industrielles, No. 267 (1935).
(4) Goodeve, C. F.: Trans. Faraday Soc. **35**, 342 (1939).
(5) Hurd, C. B., and Griffeth, R. L.: J. Phys. Chem. **39**, 1155 (1935).
(6) McDowell, C. M., and Usher, F. L.: Proc. Roy. Soc. (London) **A131**, 564 (1931).
(7) Prakash, S.: Kolloid-Z. **64**, 293 (1933).
(8) Prakash, S., and Dhar, N. R.: J. Indian Chem. Soc. **6**, 391 (1929).
(9) Prasad, M., and Desai, D. M.: J. Univ. Bombay **2**, No. 7, Part 3, 132 (1938).
(10) Prasad, M., and Hattiangadi, R. R.: J. Indian Chem. Soc. **6**, 893 (1929).
(11) Usher, F. L.: Proc. Roy. Soc. (London) **A125**, 143 (1929).

Reprinted from SCIENCE, December 31, 1943, Vol. 98,
No. 2557, page 585.

A CATALOGUE OF INSECTICIDES AND FUNGICIDES

FOR the past year we have been engaged in surveying the literature and cataloguing all materials which have been tested for insecticidal or fungicidal properties. In our opinion such a compilation will be valuable to research workers and students in the fields of agricultural chemistry, economic entomology and plant pathology, particularly since it will insure a minimum of duplication in any search for new materials. Apparently no catalogue of this nature has been prepared up to the present time.

Our search so far has yielded over 500 literature citations, from which approximately 5,000 individual insecticidal and fungicidal materials have been catalogued. It is obvious that the usefulness of such a catalogue will depend upon its completeness. While we have made every effort to cover the literature completely, there are undoubtedly journal articles, bulletins and other less widely circulated publications which have been overlooked.

In this connection, therefore, we are appealing to all workers in the fields mentioned to supply the undersigned with any unpublished data, reprints or literature citations dealing with insecticidal or fungicidal tests on specific materials, so that all such materials may be included in this catalogue. Particularly we would welcome lists of materials tested by industrial research laboratories, even though the results disclosed may be of a negative nature.

Information should include the name of the chemical compound, its formula, the name of the test organism and a general statement of toxicity. In the case of plant products, the scientific name as well as the portion of the plant used for the test should be included.

It is our intention to prepare this catalogue for publication as soon as possible. Because of the size of the undertaking, it may not be available for wide distribution, but each *bona fide* contributor will be assured of a copy. We shall be very grateful for any comments or suggestion.

DONALD E. H. FREAR

DEPARTMENT OF AGRICULTURAL
AND BIOLOGICAL CHEMISTRY,
THE PENNSYLVANIA STATE COLLEGE

SATISFACTORY WAR-TIME RATIONS FOR PHEASANT CHICKS

BY

W. J. STADELMAN, R. R. MURPHY, E. W. CALLENBACH
AND R. V. BOUCHER

Reprinted from
THE JOURNAL OF WILDLIFE MANAGEMENT
Vol. 8, No. 1, January, 1944

SATISFACTORY WAR-TIME RATIONS FOR PHEASANT CHICKS[1]

W. J. Stadelman, R. R. Murphy, E. W. Callenbach and R. V. Boucher

The Pennsylvania State College, State College, Pennsylvania

The experiment reported here was outlined (winter of 1941–42) when scarcities of ingredients used in rations for poultry and game birds were just being recognized. Critical ingredients then high in cost or difficult to obtain were dried milk products, meat scraps, and fish meals. Subsequently the quantities of these products available for livestock and poultry rations have been limited by government regulation. Thus it is difficult to maintain high feeding standards. The most promising procedure appears to be replacement of as much of the animal protein feeds as possible with more plentiful plant protein feeds.

Some of the nutritive requirements of ring-necked pheasants (*Phasianus colchicus torquatus*) have been determined. Callenbach and Hiller (1933) found their protein requirement to be about 28 per cent. Norris and others (1936) obtained greatest growth with 30 per cent of protein in the ration, but levels down to 24 per cent gave satisfactory results. The mineral tolerance of pheasants was found by Skoglund (1938) to be about the same as that of chickens. The requirements for vitamin D (Baird and Greene, 1935) and for riboflavin (Norris, 1934) for pheasants are higher than for chickens.

Wheeler (1899) found that during early stages of growth, chicks of the domestic fowl ate more and grew faster on a ration with about 40 per cent of the protein from animal sources than on other rations containing smaller percentages of animal proteins and uncorrected for phosphorus and other mineral elements. Growth was both excellent and economical when bone ash was used in rations of high plant and low animal protein content. With ducklings, however, results were poor with supplemented and unsupplemented high plant protein rations. In the work reported by Wheeler, gluten meal and O. P. (old process) linseed meal were used as plant protein replacements for animal meal.

Phillips, Carr and Kennard (1920), working with chickens, reported growth with vegetable protein feedstuffs comparable to those with meat scraps if the rations were balanced properly for mineral content.

Funk and Kempster (1940) found soybean oil meal superior to either cottonseed meal or corn gluten meal for stimulating growth of turkeys when each was fed at a 10 per cent level. More recently, Irwin and Kempster (1942) tested the value of soybean oil meal in rations for broiler chickens. Levels of soybean oil meal up to 25 per cent of the total ration produced satisfactory gains when combined with 2 per cent or more of an animal protein supplement.

Since other workers found soybean oil meal to be a satisfactory substitute for some of the animal protein concentrates for chickens and turkeys, it seemed reasonable to assume that it could serve similarly as a partial source of protein in pheasant rations. The researches here reported tested, under practical rearing conditions, pheasant starting rations that contained approximately 28 per cent of protein, largely from ingredients readily obtainable under war-time conditions.

PROCEDURE

Eight groups of 125 day-old ring-necked pheasant chicks, hatched on May 7, 1942, were reared to six weeks of age in 10×12-foot colony brooder

TABLE 1.—COMPOSITION OF THE RATIONS FED
(To each was added: salt, 0.5 lb.; cod liver oil, 0.25 lb.; and $MnSO_4$, 0.0125 lb. Each ration totalled 100.7625 pounds)

GROUP	1	2	3	4	5	6	7	8
Ground yellow corn	14.5	15.0	14.0	13.0	13.0	12.0	10.0	17.0
Wheat flour middlings	12.5	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat bran	15.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Ground oats	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Alfalfa leaf meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dry skim milk	7.5	—	—	—	—	—	—	—
Dried whey	—	10.0	10.0	10.0	10.0	10.0	5.0	5.0
Dried brewers' yeast	—	—	—	—	—	—	3.0	3.0
Meat scrap (50% protein)	10.0	20.0	16.0	12.0	8.0	5.0	3.0	12.0
Menhaden fish meal	8.0	—	—	—	4.0	2.0	2.0	3.0
Soybean oil meal	17.5	20.0	25.0	30.0	30.0	35.0	40.0	25.0
Bone meal	—	—	—	—	—	1.0	1.0	—
Ground limestone	—	—	—	—	—	—	1.0	—

---

[1] Paper No. 43, Pennsylvania Cooperative Wildlife Research Unit (U. S. and Wildlife Service, The Pennsylvania State College, Pennsylvania Game Commission, and American Wildlife Institute cooperating). Paper No. 1178, journal series, of the Pennsylvania Agricultural Experiment Station.

TABLE 2.—CHEMICAL ANALYSES OF RATIONS FED (IN PERCENTAGES)

Group	1	2	3	4	5	6	7	8
Moisture	8.43	7.93	8.48	8.56	8.31	7.86	8.89	8.92
Ash	8.34	10.62	9.74	8.60	8.12	7.97	7.88	8.41
Protein	29.31	27.56	28.38	28.25	28.31	28.56	29.00	29.06
Ether extract	5.09	4.68	4.32	3.85	4.03	4.31	3.23	4.59
Fiber	6.39	5.78	5.87	6.23	6.30	6.24	6.81	6.25
Calcium	1.72	2.08	2.46	1.65	1.48	1.31	1.40	1.67
Phosphorus	1.26	1.33	1.46	1.15	1.09	1.03	0.92	1.20
Manganese	.0076	.0068	.0074	.0076	.0070	.0084	.0068	.0076
Ca:P ratio	1.37:1	1.56:1	1.68:1	1.43:1	1.36:1	1.27:1	1.52:1	1.39:1

houses with attached sunporches. The houses were equipped with electric brooders and the usual accessory equipment for satisfactory brooding. The chicks were weighed as groups when placed in brooding quarters and wing-banded individually when 2 weeks old. They were weighed individually at 2, 4, and 6 weeks of age, on scales sensitive to 1 gram, and at each weighing were examined for symptoms of perosis and condition of feathering. Records were kept of daily mortality and weekly feed consumption.

The rations offered (Table 1) were available in suitable feeders at all times. Water and chick-size insoluble grit also were supplied. The grit was sprinkled on the mash at each feeding. The electric brooders were removed at the end of the fourth week, when the beaks of all birds were clipped to control cannibalism.

The ration fed Group 1 contained 17.5 per cent of soybean oil meal in combination with dry skim milk, meat scraps and menhaden fish meal; this has been recommended for general use by Stadelman, Murphy, Callenbach and Boucher (1941). In each of the seven other rations, dry skim milk was replaced by dried whey or a combination of dried whey and yeast. The fish meal was omitted entirely from the rations of Groups 2, 3 and 4, the protein being supplied by variable proportions of meat scraps and soybean oil meal. In all other rations the protein carriers—menhaden fish meal, meat scraps, and soybean oil meal—were used in various combinations. The total protein content of all rations was calculated to be 28 per cent. Exact chemical analyses are given in Table 2.

RESULTS AND DISCUSSION

All the rations tested produced satisfactory game birds. The average body weights (Table 3) were normal for ring-necked pheasants at the ages indicated. At six weeks of age, Groups 1, 5, 6 and 8 were slightly heavier than the other four, but none of the differences is statistically significant.

A factor of considerable importance in game bird production is the quantity and quality of feather development and maintenance, described here as feather condition. In this respect Group 7 ranked first, closely followed by Group 1. The feathering of Groups 2 to 6 was about the same. The birds of Group 8 were poorest in feather condition at six weeks of age, due almost entirely to feather picking. Birds in all groups were feathered sufficiently for release.

TABLE 3.—AVERAGE INDIVIDUAL BODY WEIGHTS (GRAMS).

Group	1	2	3	4	5	6	7	8
Day-old	21	21	21	21	21	21	21	21
2 weeks	63	62	62	61	64	63	63	69
4 weeks	167	152	156	155	164	160	154	175
6 weeks	296	279	282	275	297	293	269	294

TABLE 4.—FEED CONSUMPTION PER 100 BIRDS (POUNDS)

Group	1	2	3	4	5	6	7	8
Week								
1	11.8	10.9	10.8	11.2	11.0	11.4	11.4	11.7
2	19.4	19.8	19.7	19.3	20.0	19.5	19.1	18.7
3	25.6	25.7	26.9	27.0	29.3	27.6	26.9	26.4
4	37.3	36.6	37.8	38.1	36.2	39.0	39.2	38.1
5	43.1	43.0	44.0	44.6	46.9	44.7	43.0	43.6
6	50.2	52.7	54.4	55.0	56.6	59.2	53.6	53.0
Totals	187.4	188.7	193.6	195.2	200.0	201.4	193.2	191.5

TABLE 5.—POUNDS OF FEED REQUIRED TO PRODUCE ONE POUND OF GAIN

Group	1	2	3	4	5	6	7	8
Period, weeks								
0–2	3.4	3.4	3.4	3.4	3.3	3.4	3.3	2.9
2–4	2.7	3.1	3.1	3.2	3.0	3.1	3.3	2.8
4–6	3.3	3.4	3.6	3.8	3.5	3.5	3.8	3.8
0–4	2.9	3.2	3.2	3.2	3.1	3.2	3.3	2.8
0–6	3.1	3.3	3.4	3.5	3.3	3.4	3.5	3.2

TABLE 6.—WEEKLY MORTALITY PER GROUP OF 125 CHICKS (PERCENTAGES)

Group	1	2	3	4	5	6	7	8
Week								
1	2.4	0.8	3.2	0.8	0.8	3.2	0.0	0.0
2	0.0	0.8	0.0	2.4	0.8	4.0	0.0	0.0
3	1.6	0.8	0.0	0.0	0.0	0.0	0.8	0.0
4	0.0	0.8	0.0	0.8	0.0	0.0	0.0	0.0
5	0.8	0.0	0.0	0.8	0.8	1.6	0.0	0.8
6	0.0	0.8	0.0	0.0	1.6	0.0	0.8	0.8
Total	4.8	4.0	3.2	4.8	4.0	8.8	1.6	1.6

The variation in total consumption of feed was very slight among groups (Table 4), the widest being 7 per cent between Groups 1 and 6. None of the differences is significant. Group 1 was most efficient in feed utilization, but variations between groups were not of sufficient magnitude to be of primary importance; Groups 4 and 7 were least efficient (Table 5).

Mortality by weekly periods is given in Table 6. The mortality in Group 6 during the second week was caused by crowding in corners of the pen. Only two chicks were lost by cannibalism, both in Group 5 during the sixth week. Livability in all groups was quite satisfactory, being best in Groups 7 and 8 where only two chicks each were lost during the entire six weeks.

The quality of the birds in all groups was excellent. There were no signs of perosis or dietary deficiency in any group and no stunted birds were found at the end of the experiment.

The results indicate that in pheasant starting rations soybean oil meal can replace large proportions of the usual animal protein supplements and that dried whey or a combination of dried whey and brewers' dried yeast can replace dry skim milk.

SUMMARY

Eight groups of 125 ring-necked pheasant chicks were reared to six weeks of age on as many different rations. Records were kept of daily mortality, weekly consumption of feed, bi-weekly weights, and feathering condition.

All the rations tested, including one with 40 per cent of soybean oil meal, were excellent for rearing, indicating a much wider range of tolerance with respect to sources of proteins than anticipated.

The selection of any one of the rations reported should be based primarily on availability and relative cost of the ingredients specified.

LITERATURE CITED

BAIRD, F. D., and D. J. GREENE. 1935. The comparative vitamin D requirements of growing chicks, turkeys and pheasants. Poul. Sci., **14**: 70–82.

CALLENBACH, E. W., and C. A. HILLER. 1933. The artificial propagation of ring-necked pheasants. Pa. Agr. Expt. Sta. Bul., 299.

FUNK, E. M., and H. L. KEMPSTER. 1940. The use of vegetable protein concentrates for raising turkeys. Missouri Agr. Expt. Sta. Bul., 414.

IRWIN, M. R., and H. L. KEMPSTER. 1942. The value of soybean oil meal in broiler rations. Missouri Agr. Expt. Sta. Bul., 441.

NORRIS, L. C. 1934. Some studies of nutritive requirements of pheasants. Amer. Game Conf. Trans., **20**: 304–310.

NORRIS, L. C., L. J. ELMORE, R. C. RINGROSE, and G. BUMP. 1936. The protein requirement of ring-necked pheasant chicks. Poul. Sci., **15**: 454–459.

PHILLIPS, A. G., R. H. CARR, and D. C. KENNARD. 1920. Meat scraps versus soybean proteins as a supplement to corn for growing chicks. Jour. Agr. Res., **18**: 391–398.

SKOGLUND, W. C. 1938. The mineral requirements of ring-necked pheasant chicks. Unpublished data.

STADELMAN, W. J., R. R. MURPHY, E. W. CALLENBACH, and R. V. BOUCHER. 1941. Dry skim milk in ring-necked pheasant chick rations. Game Breeder and Sportsman, **46**: 102, 103, 114–116.

1941. A comparison of pheasant starting rations under practical commercial conditions. Pa. Game News, **12**(5): 4, 5, 28, 29.

WHEELER, W. H. 1899. Animal food for poultry. N. Y. Agr. Expt. Sta. Ann. Rept., **18**: 75–124.

BULLETIN 463 FEBRUARY 1944

Deposition and Retention of Sprays. III.

Apparatus and Methods for Laboratory Spraying

The Pennsylvania State College
SCHOOL OF AGRICULTURE
Agricultural Experiment Station
State College, Pennsylvania

DIGEST

This paper presents a laboratory method for the study of the deposition and retention of insecticide and fungicide sprays. In this method synthetic surfaces attached to a rotating turntable are sprayed with an atomizer type sprayer, to which is attached an overhead reservoir containing the spray suspension. The effects of time, pressure, concentration of toxicant, and type of sprayer were investigated in connection with the performance of the apparatus, and the method finally evolved was found to give accurate and reproducible results. The plates sprayed by this method may be readily subjected to artificial weathering tests, and retention coefficients calculated. Typical results are given.

Deposition and Retention of Sprays. III.

Apparatus and Methods for Laboratory Spraying.*

Donald E. H. Frear †

THE MOST IMPORTANT CONSIDERATIONS involved in the performance of any insecticide or fungicide, apart from the inherent toxicity of the material used, are the amounts of toxicant deposited and retained following application. A highly toxic material may fail to show insect or disease control under practical conditions because of low initial deposition or poor retention, or a combination of the two. Conversely, a substance of less toxicity may produce satisfactory control because of its superior qualities of deposition and retention.

Since this is true, it follows that an increase in these desirable qualities should be one goal toward which research workers, particularly those in the field of insecticide and fungicide chemistry, should strive. Materials long used may be improved, and substances discarded because of unsatisfactory performance may be rendered useful.

Unfortunately, the problem of how to improve the qualities of deposition and retention of insecticides and fungicides is neither simple nor easy. There are two obvious ways to study such a question; first, by a routine testing of a large number of possible materials in all manner of combinations, a tremendous undertaking; or second by the application of knowledge of the fundamental processes involved to a specific problem. The latter approach certainly is the more scientific one. However, in spite of a great deal of work on the subject, knowledge of the fundamental principles involved in spray deposition and retention is very limited. This is due in some measure, at least, to the fact that field experiments as usually conducted are lengthy and costly.

Field experiments, although they may be expected to yield results of a definitely practical nature, require nearly an entire growing season, as well as rather costly and laborious chemical analysis. Such studies have been reported by a number of workers, including the author (3 and 4). Laboratory studies on spray deposition and retention are quicker and more readily carried out, although they do not always correlate with the results secured in the field (6 and 14). Field studies, even though closely controlled, are subject to a number of unknown and uncontrollable variables, while laboratory studies may be carried out with a much higher degree of precision. For this reason laboratory studies are to be preferred in any attempts to investigate the more fundamental aspects of the problem.

---

* Authorized for publication January 11, 1944.

† Associate Professor of Agricultural and Biological Chemistry.

A number of ingenious devices and techniques have been proposed for laboratory spray studies. None of these, however, exactly met all of the requirements of the investigations which the author had in mind. Therefore, about eight years ago work was begun on apparatus for laboratory spraying which would more nearly meet the specific needs of spray deposit investigations. During the following years the apparatus has been used for deposition and retention studies on both insecticides and fungicides. As experience with the apparatus increased, changes in the design were made, so that the present apparatus now represents the accumulated experience of a number of workers[1] over a period of approximately eight years. It is the purpose of this paper to describe this apparatus, and to give some information as to its performance and possibilities. It is hoped that the presentation of this information will be of value to other investigators in the field.

GENERAL CONSIDERATIONS

Definitions.—Before entering into a discussion of the theoretical aspects of deposition and retention, it may be wise to define the terms used. In the past there has been considerable confusion of terms used in this type of investigation. The terminology used in this paper conforms in general to that suggested by the Committee on Standardization of Fungicidal Tests of the American Phytopathological Society *(8)*, except for the term *retention.* Retention has been used in the earlier papers in this series to denote the property of a spray deposit to resist removal by weathering and will be used throughout this present paper in reference to *tenacity,* the term preferred by the committee.

Theoretical considerations.—Probably the best way to discuss the processes involved in the deposition and retention of a spray is to use a concrete example. A suspension of the finely divided solid is prepared and placed in the spray tank. By the proper application of a mechanical force this suspension is forced through an orifice under pressure and at this point is broken up into droplets of various sizes. The amount of the flow and the size of the droplets will depend on the amount of force (pressure) applied to the liquid, the size and conformation of the nozzle and orifice, the physical properties of the liquid, surface tension, viscosity, etc., as well as the temperature and humidity of the air.

Depending on the particle size of a suspended material, such as lead arsenate, each droplet may contain one or more particles of the suspended solid. During their passage through the air these droplets may lose water through evaporation unless the humidity is 100 per cent. Some of the smaller droplets may evaporate completely, leaving the solid particles of suspended material free to float away. A certain percentage of the droplets reach the target toward which they were directed. Here the behavior of the drop is further influenced by the character of the surface against which it strikes. In a remarkable series of photographs, Upholt and Hoskins *(13)* have illustrated the changes in conformation of droplets at the moment of impact and immediately thereafter. As the drop of liquid strikes the solid surface there is usually a more or less pronounced rebound, resulting in spattering if the drop is of sufficient size. Following this there is a subsidence, during which time the drop assumes a characteristic shape, depending on the composition of the liquid and its physical properties and the character of the surface. The extent to which a drop spreads on the surface is the resultant of these forces, the surface energy of the solid, the surface tension of the liquid, and the wetting and spreading ability of the liquid. Eventually these come to equilibrium and form a drop pattern characteristic for the liquid and surface involved.

If the liquid wets the solid on which it was deposited, it tends to spread out in a thin film, with the angle of contact approaching zero. If the liquid does not wet the surface, discrete droplets, more or less spherical in shape, rest on the surface without wetting it. In this case the contact angle is 180 degrees. The surface tension of the liquid tends to pull drops into the shape having the smallest surface area, the sphere. Intermediate between no wetting and complete extension of the drop on the surface there are, obviously, many degrees of wetting and spreading.

The droplet of spray suspension, now at rest on the surface to which it has fallen, normally begins to evaporate at once. The speed with which the liquid phase is evaporated depends upon the humidity of the atmosphere, the temperature, the vapor pressure of the liquid, and the free surface exposed. As evaporation proceeds, the ratio between suspended solids to liquid becomes progressively greater. In cases where the liquid phase contains dissolved substances as well as suspended material, the opportunities for chemical reactions are greatly increased. Gases may have been dissolved in the spray droplets in their passage through the air, and these may function as chemical reactants at this time.

When evaporation becomes complete, the dry residue consists of the solids which were originally suspended or dissolved in the liquid phase. These particles are retained by the surface upon which they have been deposited with varying degrees of tenacity. Among the factors which determine the degree to which these particles adhere are the size of the particles, their shape, the nature of the particle surface, and the nature of the surface (substratum) upon which they are deposited. When subjected to weathering, which includes mechanical abrasion, rainfall, dews, and atmospheric gases, solubility and chemical reactivity play an important part, along with the other factors mentioned in determining the amount of residue retained upon the surface. Moore *(10)* has indicated that in some cases electrical charges on the particles and on the substratum may have a significant effect in determining adherence.

---

[1] A number of persons have given freely of their advice and assistance in the development of this apparatus. Special credit should be given to H. N. Worthley, H. W. Thurston, Jr., H. J. Miller, H. L. King, James H. Stewart, R. M. Bovard, and John B. Harry.

DESCRIPTION AND TESTS OF NEW APPARATUS

In the following pages is presented a description of the apparatus and method as used at present in this laboratory. As mentioned previously, this apparatus and technique represent the results of a number of trials over a period of years and is the expression of ideas suggested by a number of workers. An attempt will be made to characterize the performance of the apparatus and to demonstrate the accuracy and reproducibility of the laboratory method as a whole. It is hoped that this information will be of some use to other research workers and will stimulate further work on the subject of spray deposition and retention.

Apparatus.—Two views of the completed apparatus are given in figures 1 and 2. The principal parts are: (1) A rotating circular table, 4 feet in diamter, mounted on a central vertical shaft, and rotated at a speed of 14 revolutions per minute by a synchronous motor (one sixteenth h. p.) connected by a belt through a speed reducer which is geared directly to the shaft. The reducing gear, motor, and bearings for the table axle are bolted firmly to an angle iron frame which forms a base to give the assembly complete rigidity. The turntable is made of one piece of $\frac{3}{4}$ inch plywood cut in a circle 4 feet in diameter. (2) A series of 24 wooden blocks arranged around the periphery of the wheel on its top surface. These blocks are beveled so as to form a complete circle, with their outer surfaces cut to

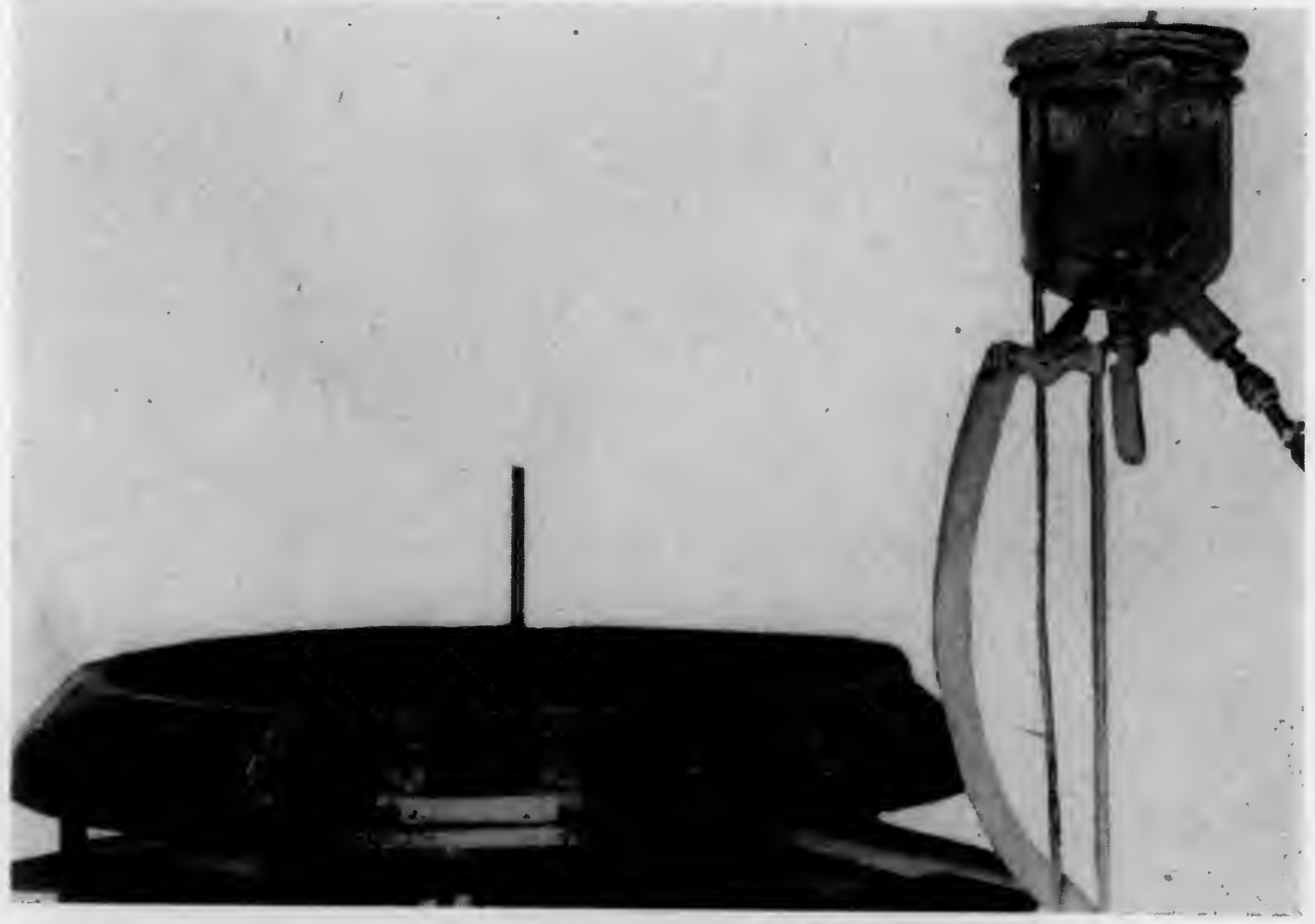
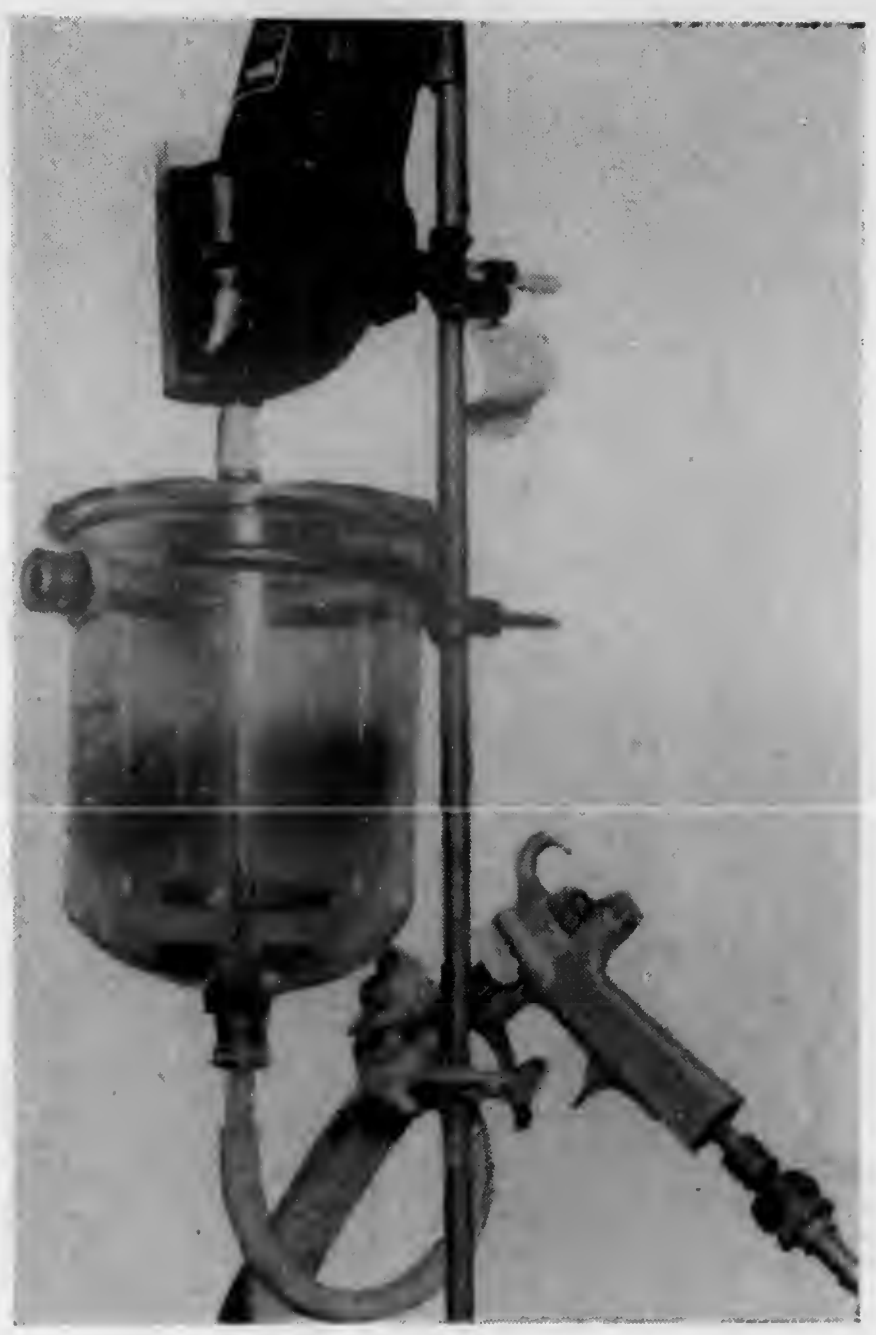


Fig. 1.—Complete spraying apparatus, showing relation of rotating turntable to atomizer sprayer. The motor and driving gears are located underneath the turntable and are not visible.

Fig. 2.—Close-up of atomizer sprayer assembly, showing reservoir above atomizer, stirring motor, and connections. The large flexible metal hose leading down from the atomizer is used to catch the spray stream when cleaning the atomizer.



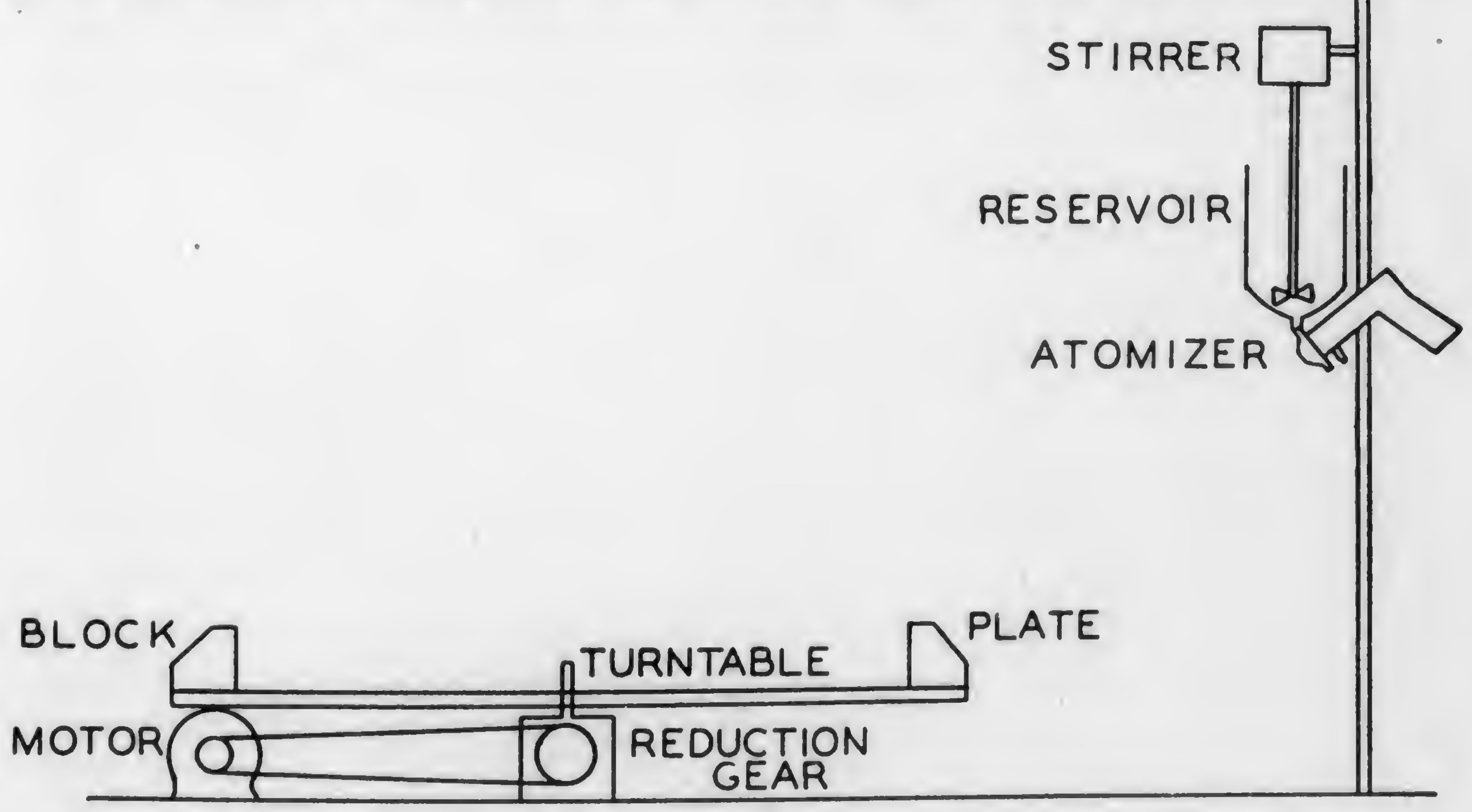


Fig. 3.—Diagram showing the arrangement of the essential parts of the laboratory spraying apparatus.

present surfaces approximately 5 inches square at an angle of 45 degrees to the table surface. Figure 3 shows a vertical section through the center of the turntable.

(3) A spray nozzle, mounted so that the spray stream is directed at right angles to the beveled surface of the block. The types of nozzles tested will be discussed in more detail later. The surface to be sprayed is attached to the beveled surface of the wheel, sprayed for the required length of time, dried, removed, and the deposit analyzed. For retention studies the dried deposit is subjected to artificial rainfall usually applied through the same nozzle used to apply the deposit. This also will be discussed in more detail later.

Types of experimental surfaces used.—A wide variety of surfaces, both natural and synthetic, have been used for laboratory studies. Many workers have used living plant surfaces, particularly leaves and fruits. In such tests, the plants or fruits to be tested are usually rotated in the stream of spray and the residue determined by mechanical, chemical or biological means. Swingle, Phillips, and Gahan *(12)*, for example, used living leaves and Newcomber *(11)* used apple fruits. Many other workers have used synthetic surfaces. McCallen and Wilcoxon *(7)* used glass microscope slides, Dawsey et al. *(2)* used wax-coated mica plates, Borden and Hensill *(1)* wax-coated bottles, and Horsfall et al. *(5)* and a number of English workers have used glass plates covered with cellulose nitrate.

Since there is great variation among natural leaf surfaces, from species to species and even from leaf to leaf on the same plant, such surfaces introduce an undesirable variable in laboratory spraying experiments. The use of some synthetic surface which can be reproduced accurately and consistently would appear to offer great advantages, in spite of the fact that such a surface probably does not correspond exactly to any known natural surface.

In the tests planned for this apparatus it was necessary to have relatively large areas of sprayed surfaces for subsequent analyses, thus ruling out microscope slides. Preliminary tests were made on glass plates, and on several types of synthetic organic surfaces. Glass plates, when chemically clean, were very easily wetted, and hence did not correspond to most natural surfaces. Cellulose acetate and cellulose nitrate (celluloid or Pyralin) sheets were also extensively tested, and the latter was found to be the most suitable material. The droplet pattern on sheets of this substance were comparable to those observed on many natural surfaces; it was available in quantity at a reasonable cost, and lots secured at different times were found to have very uniform properties. The commercial designation of the material used for all subsequent tests was *colorless Pyralin, 0.0125 inch thick.* It is available in sheets 20 by 50 inches from the E. I. du Pont de Nemours and Company, and is cut into plates 5 by 5 inches just before use. Rubber gloves are always used in handling and cutting the sheets to avoid contamination. When ready for analysis, the sprayed plates are trimmed to exactly 10 by 10 centimeters, and all analyses are expressed on the basis of 100 square centimeters of surface.

Types of sprayers used.—Two general types of sprayers have been used for laboratory studies on deposit. The first more widely used type is based on the well-known atomizer principle. Most workers using such a sprayer for laboratory studies have disregarded certain rather obvious sources of error involved. Commonly the receptacle containing the liquid to be atomized is below the orifice through which it is discharged. In the case of a homogeneous liquid, of course, the liquid discharged has the same composition as that in the container below. With a solid suspended in the liquid however, the situation is not so simple. Rarely, if ever, are the specific gravities of the suspended phase and the suspending phase the same. In the case of most solids usually used for spraying, the specific gravity is greater than that of the suspending liquid (commonly water), and hence the particles have a tendency to settle to the bottom of the container. They may be kept in a more or less uniform state of suspension by the use of some sort of mechanical stirrer. This is usually done. However, as soon as the suspension is drawn into the delivery tube it is removed from the sphere of action of the stirrer, and the heavy particles begin to settle at once due to the force of gravity. Acting against this force is the upward movement of the suspension caused by the pull from above, which tends to draw both phases upward. The solid particles, however, move upward less rapidly than the liquid phase. This may be observed readily in an all-glass apparatus. The result of this phenomenon is a fractionation: relatively more of the liquid phase and less of the solid phase are discharged than are present in the container. This is shown conclusively by the figures in table 1.

The figures in this table were secured as follows: one liter of a suspension of lead arsenate was prepared and, while thoroughly stirred with a mechanical stirrer, a portion was sprayed through an adjustable atomizer type spray gun (a commercial gun made for automobile painting) with an air pressure of 80 pounds per square inch. Approximately 500 milliliters of the suspension was sprayed out and collected in a tall cylinder so designated as to catch all of the spray discharged. Samples of both the collected spray and the residue remaining in the original container were thoroughly stirred, and 100 ml aliquots withdrawn as rapidly as possible with a pipette. These were transferred to weighed dishes, dried, and the residue weighed. The suction tube was 9 inches long, and extended nearly to the bottom of the 1.5 liter beaker used to hold the spray suspension.

The figures in table 1 indicate that when using the reservoir below the orifice, the composition of the spray discharged may be decidedly different from that of the original suspension, the discharged spray having on the average only 90.9 per cent as much suspended solids as the original suspension. Other tests with various materials have demonstrated that this error is usually present in this type of apparatus.

Table 1.—Concentration of lead arsenate in the collected discharge from the atomizer compared with the concentration in the suspension remaining in the reservoir; reservoir below atomizer.

GRAMS $PbHAsO_4$ PER LITER OF SPRAY SUSPENSION	GRAMS $PbHAsO_4$ IN COLLECTED SPRAY (as calculated from aliquot)	GRAMS $PbHAsO_4$ IN SPRAY IN RESERVOIR (as calculated from aliquot)	RELATIVE PROPORTION OF $PbHAsO_4$ (original suspension = 100) Collected spray	Reservoir
3.6000	3.348	3.605	93.0	100.1
3.6000	3.320	3.565	92.2	99.0
3.6000	3.499	3.587	97.2	99.6
3.6000	3.466	3.619	96.3	100.5
10.8000	9.616	11.025	89.0	102.1
10.8000	9.580	11.221	88.7	103.9
10.8000	9.212	10.827	85.3	100.3
10.8000	9.209	11.134	85.3	103.1
MEAN			90.9	101.1

Some workers have placed the reservoir above the orifice of the atomizer, thus reversing the gravitational effect. This arrangement was tried, samples being taken in the same manner as those previously described. The results are given in table 2. With the reservoir above the atomizer the mean toxicant concentration in the spray was almost exactly the same as when the suspension was made up, the average being 100.7 per cent. The suspension remaining in the reservoir, however, showed only 95.3 per cent of the original concentration. The difference probably represents a small amount of settling in the reservoir.

The discharge of spray appeared to be more uniform from run to run when the reservoir was placed above the orifice of the atomizer gun. This is shown in figure 4, in which the time required to discharge 500 ml of water was measured at different adjustments of the atomizer gun used. This adjustment regulated the ratio of liquid to air in the discharge, and was made by turning a knurled knob on the gun. Time measurements were made using the reservoir above and below the orifice. The variability of the time measurements in curve A (reservoir below) is noticeably greater than in curve B (reservoir above). The rate of discharge was much more rapid, naturally, when the reservoir was above the orifice.

When using a reservoir with a diameter of 15 cm the withdrawing of 1 liter of liquid lowered the liquid level approximately 5 cm. Extensive tests have demonstrated that this change in level produced a negligible change in rate of discharge; for extreme precision, however, the liquid level may be adjusted to correct for the drop in pressure head or a constant level device may be used.

Table 2.—Concentration of two suspended toxicants in the collected discharge from the atomizer compared with the concentration in the suspension remaining in the reservoir; reservoir above atomizer.

GRAMS TOXICANT ADDED PER LITER OF SPRAY SUSPENSION	GRAMS TOXICANT IN COLLECTED SPRAY (as calculated from aliquot)	GRAMS TOXICANT IN SPRAY IN RESERVOIR (as calculated from aliquot)	RELATIVE PROPORTION OF TOXICANT (original suspension = 100) Collected spray	Reservoir
$PbHAsO_4$— 3.6000	3.60	3.42	100.0	95.0
	3.59	3.40	99.7	94.4
	3.68	3.44	102.2	95.6
	3.70	3.37	102.8	93.6
$PbHAsO_4$—10.8000	10.76	10.60	99.6	98.1
	10.74	10.46	99.4	96.9
	11.05	10.41	102.3	96.4
	11.01	10.25	101.9	94.9
Basicop — 3.6000	3.60	3.51	100.0	97.5
	3.52	3.42	97.8	95.0
	3.68	3.31	102.2	91.9
	3.62	3.37	100.6	93.6
Basicop —10.8000	10.91	10.53	101.0	97.5
	10.98	10.26	101.7	95.0
	10.74	10.28	99.4	95.2
	10.88	10.14	100.7	93.9
MEAN			100.7	95.3

With the adjustable atomizer, which is commonly employed in all studies with this apparatus, it is possible to standardize the delivery volume within narrow limits. It has become standard practice to check the delivery volume for a given period of time before each spraying, as well as to use the same volume of spray suspension in the reservoir in order to eliminate possible variations in the method.

Having determined that the atomizer type sprayer with the reservoir above the orifice discharged a spray having the same composition as the original mixture, a comparison between this type of sprayer and a positive pressure sprayer was made. The positive pressure sprayer is used almost exclusively in commercial operations, and all field sprayers are based on this principle. A miniature pressure sprayer was secured, and fitted with a 10-gallon tank. The tank was equipped with a mechanical agitator which gave thorough agitation at all times. The pump, a single cylinder model, developed a maximum of 300 pounds per square inch pressure, but in these tests was used at 100 pounds per square

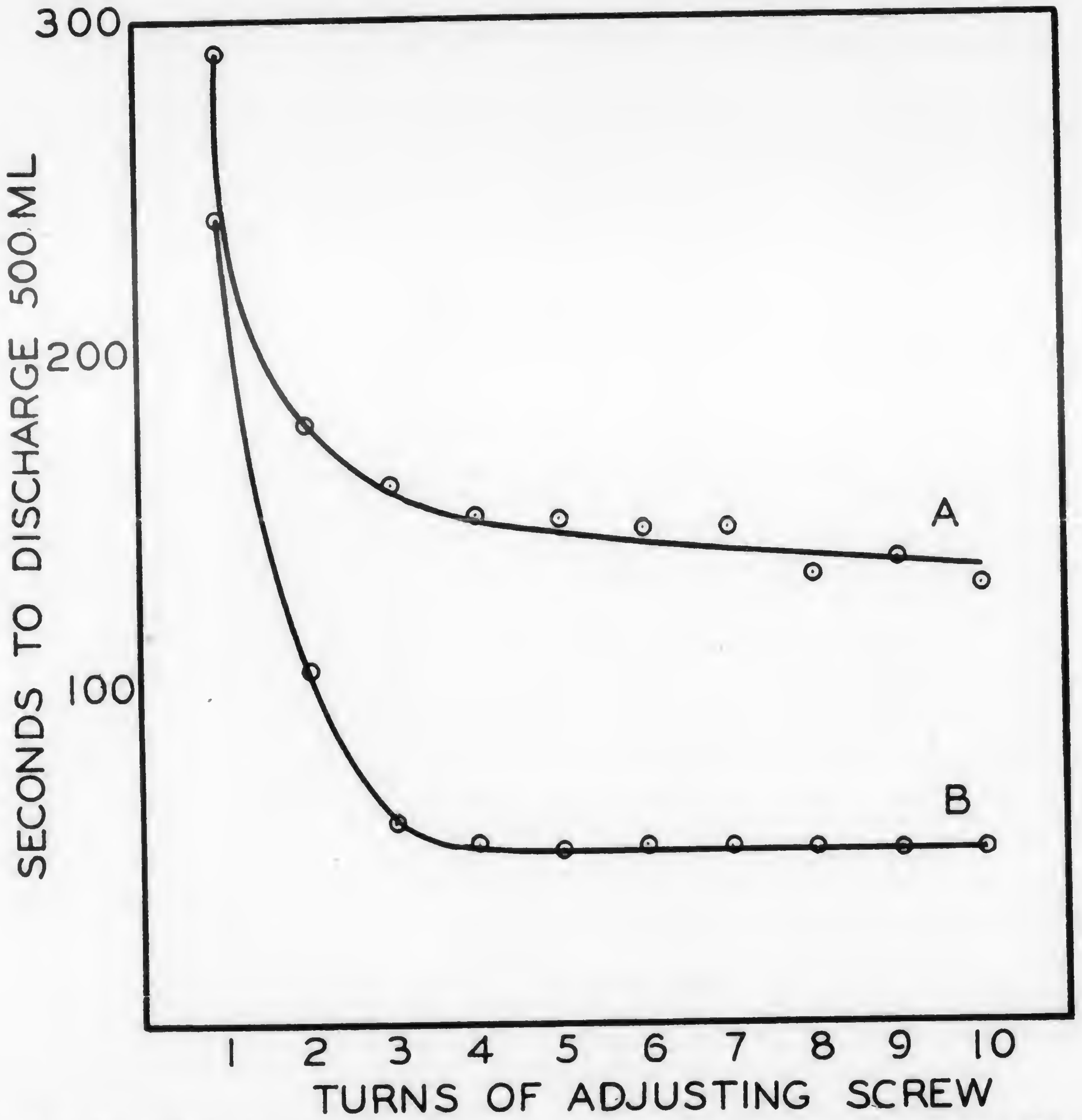


Fig. 4.—Relation between discharge time and atomizer adjustment. Line A represents the results with reservoir below atomizer; line B, with reservoir above atomizer. Note lack of consistency in line A.

inch. The nozzle was a right angle, misting type with an orifice 0.037 inch in diameter, delivering 0.094 gallons per minute at 100 pounds per square inch pressure[2].

Using this outfit, a series of Pyralin plates were sprayed with a suspension of 4 pounds of BORDOW per 100 gallons. Five plates were sprayed one revolution of the wheel, 5 for two revolutions, 5 for three revolutions, and 5 for four revolutions on six different days. The plates were analyzed for copper, and the standard deviations calculated. For comparison, the same procedure was repeated using the atomizer type spray gun mentioned previously, using 80 pounds per square inch air pressure. The results are given in table 3. Each figure represents the average of five plates, although the standard deviation and group averages were calculated from the individual data.

From table 3 it is apparent that the atomizer sprayer gave much more uniform and reproducible data. The standard deviation of the atomizer sprayed plates varied from 4.6 to 10.9 per cent of the mean values; for the positive pressure apparatus the standard deviations ranged from 10.8 to 18.4 per cent of the mean values.

In order to compare the reproducibility of this method of spraying with the settling tower method of McCallan and Wilcoxon *(7)*, coefficients of variation were calculated in the same manner as reported by these authors. The coefficient of variation between replicate tests (different times) on the rotating table was 69.3 for the positive pressure apparatus and 16.3 for the atomizer. This latter figure is approximately equal to the similar value obtained by McCallan and Wilcoxon for their settling tower technique. The coefficient of variation between plates sprayed at the same time was 5.8 for the positive pressure, and 5.4 for the atomizer sprayer, figures which are again approximately the same as those obtained by the settling tower method. This method of statistical analysis further indicates the superiority of the atomizer as a spraying apparatus over the positive pressure type.

On the basis of these results, the atomizer type sprayer with the reservoir above the orifice was adopted as giving the most consistent and reproducible results.

Table 3.—Uniformity tests conducted with 4 pounds Bordow per 100 gallons of spray suspension; average amounts of copper deposited on 5 plates.

	1 REVOLUTION (micrograms per plate)	2 REVOLUTIONS (micrograms per plate)	3 REVOLUTIONS (micrograms per plate)	4 REVOLUTIONS (micrograms per plate)
		Positive Pressure Pump		
	120	176	236	293
	108	163	225	289
	83	133	187	267
	73	133	161	227
	83	150	200	255
	93	151	193	208
True mean	93	151	203	256
		Atomizer		
	98	164	234	287
	96	192	225	287
	98	175	245	311
	125	163	245	314
	106	168	236	294
	104	156	218	288
True mean	104	170	234	297
Standard Deviation:				
Positive Pressure	17.2	16.3	26.0	34.4
Atomizer	11.3	12.2	13.4	13.8

---

[2] Nozzle type T-68037, manufactured by Spray Engineering Co., Somerville, Mass.

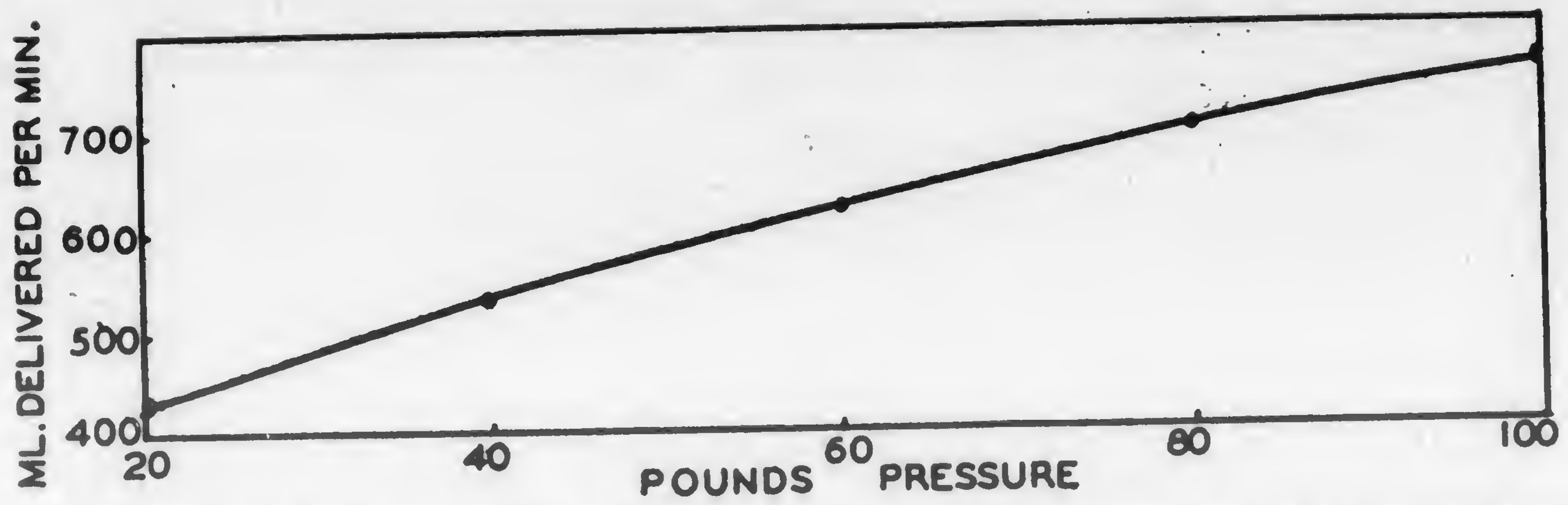


Fig. 5.—Relation between discharge rate and air pressure using atomizer sprayer with reservoir above outlet.

Effect of air pressure.—In order to ascertain the relation between air pressure and delivery of spray, the atomizer gun was calibrated at different pressures by quantitatively collecting the discharge and measuring its volume. This calibration is shown in figure 5. Water was used as the liquid in this test. It may be seen that the volume of liquid was directly related to the air pressure, although the line is slightly curved, indicating a lower efficiency as the pressure increased.

A series of Pyralin plates sprayed at different pressures indicated that increased delivery does not necessarily result in increased deposit. This is shown in figure 6. In this test the plates were sprayed with a suspension of 3 pounds of Bordow per 100 gallons for 3 revolutions of the rotating table. It may be seen from this figure that the amount of toxicant deposited on the plates increased rather regularly up to and including 80 pounds per square inch air pressure, and then decreased at 100 pounds pressure. Apparently this phenomenon was caused by a deformation of the spray cone at the higher pressure. Eighty pounds per square inch was selected as standard for all subsequent experiments, since it appeared to give maximum deposit on Pyralin plates.

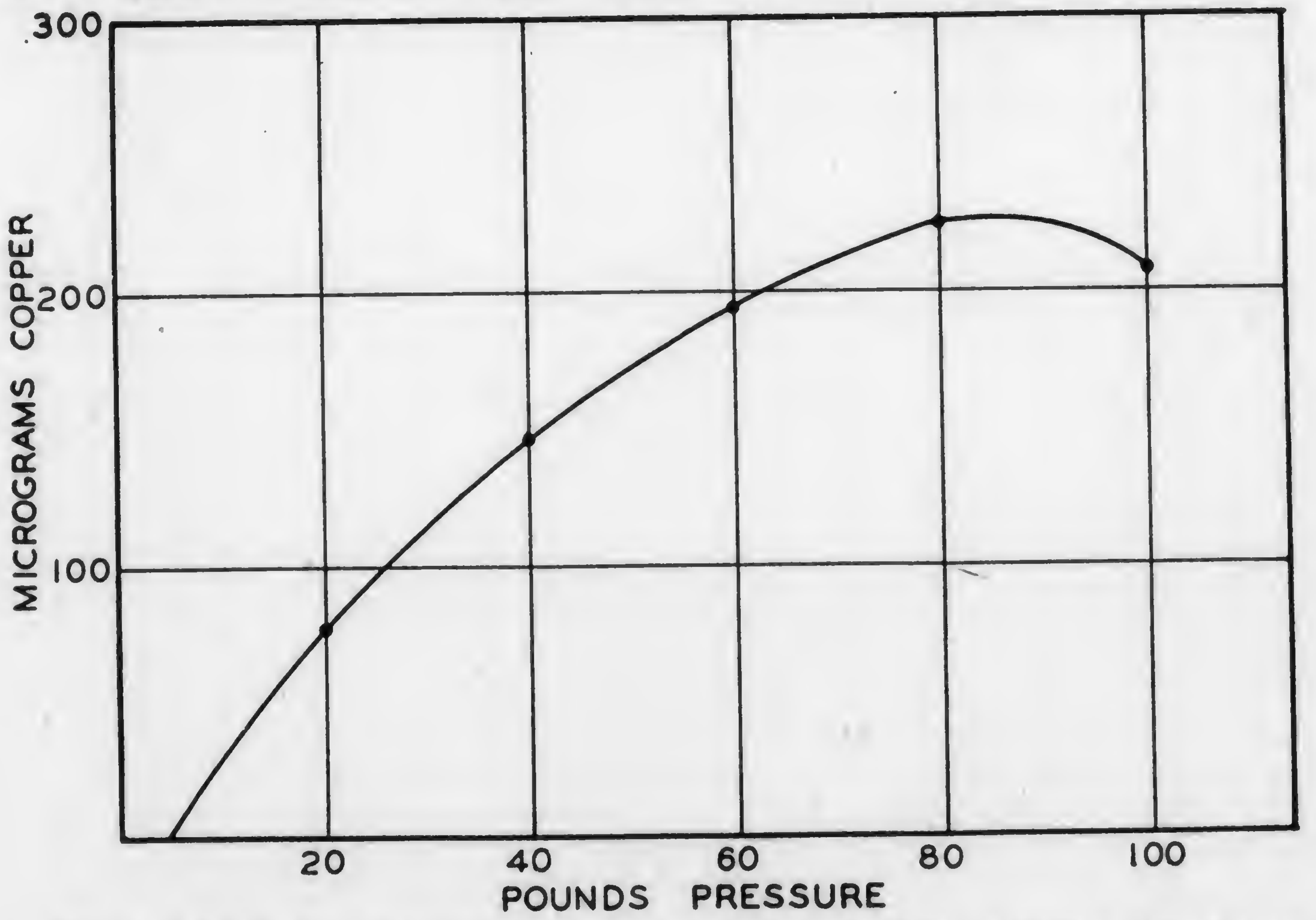


Fig. 6.—Relation between air pressure and amount of spray deposit on *Pyralin* plates.

Effect of concentration.—The effect of concentration of toxicant in the spray suspension on the amount deposited upon Pyralin plates is shown in figure 7. Bordow was used as the suspended material in these tests. There appears to be a regular increase in deposit with increases in concentration, but, as in the case of the pressure changes, the response is not linear; that is, 2 pounds of toxicant per 100 gallons gave a deposit of approximately 130 micrograms of copper per plate, while 4 pounds gave only 240 micrograms. The curve, however, is very regular. Since the concentration of toxicant may be varied to suit the type of experiment, this factor has not been standardized, but it has been the custom

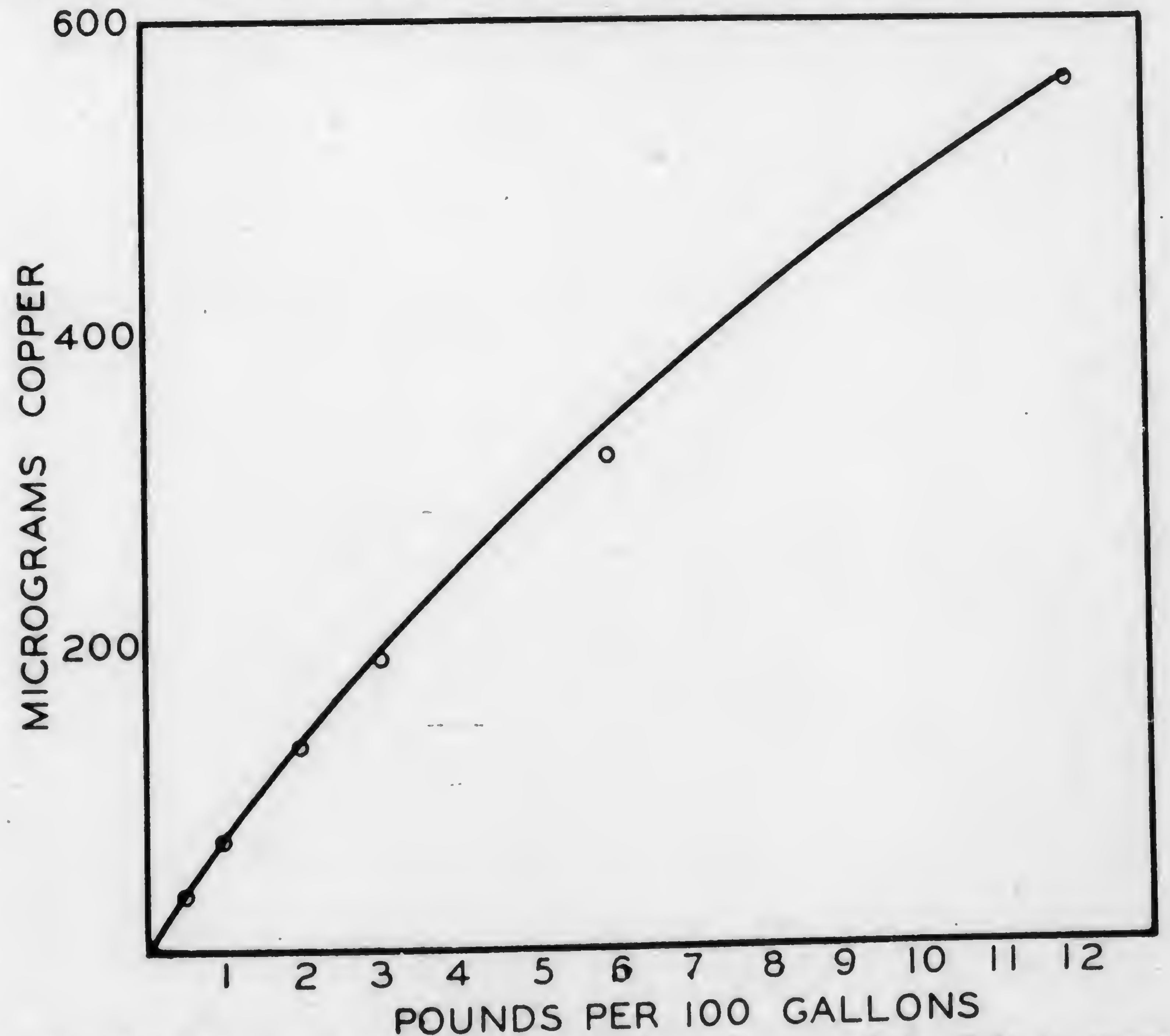


Fig. 7.—Relation between concentration of suspended solids in spray mixture and amount of spray deposited on *Pyralin* plates.

to use most materials at concentrations normally used in the field—usually 2, 3, or 4 pounds per 100 gallons. Lots for laboratory spraying are usually made in 1- or 2-liter quantities.

Effect of time of spraying.—There has been considerable discussion among workers in the field as to the optimum length of time for spraying. To a certain extent this depends upon the problem investigated. For example, if one sprays to the point of drop coalescence, regardless of the length of time required, there is a tendency to mask the effect of added wetting and spreading agents. The determination of this point, too, is rather arbitrary, and depends upon the judgment of the operator. Others make a practice of spraying to the point of run-off following the procedure normally used in field tests. Here again the judgment of the operator may cause differences in the final result. In figure 8 it is possible to see what happens when Pyralin plates are sprayed for different lengths of time. In most of the work done using the complete apparatus, one revolution of the table has been used as a measure of time, rather than seconds or minutes. Using the synchronous motor, the speed of the turntable is constant, and in actual practice one revolution requires approximately four seconds.

In figure 8, line A represents the course of deposit on Pyralin plates sprayed with a suspension containing 3 pounds of BORDOW per 100 gallons. This material has the property of forming discrete droplets upon the sprayed surface without wetting it, rather than a continuous film. When sprayed on a surface, therefore, the droplets grow larger and larger as the spraying time is increased, up to the point where they become so large that they run off due to gravitational pull. In figure 8 this process is readily seen, the deposit increasing in a regular manner up to 6 revolutions. On the seventh revolution some run-off occurred, the deposit decreasing slightly for this reason. During the eighth and ninth revolutions this process continued, until practically all of the large droplets had left the plate through run-off, leaving large areas of the plate free from spray. These areas, consisting mainly of dry plate surface, accepted additional small droplets of spray, and the process reached an equilibrium, run-off being equal to deposit.

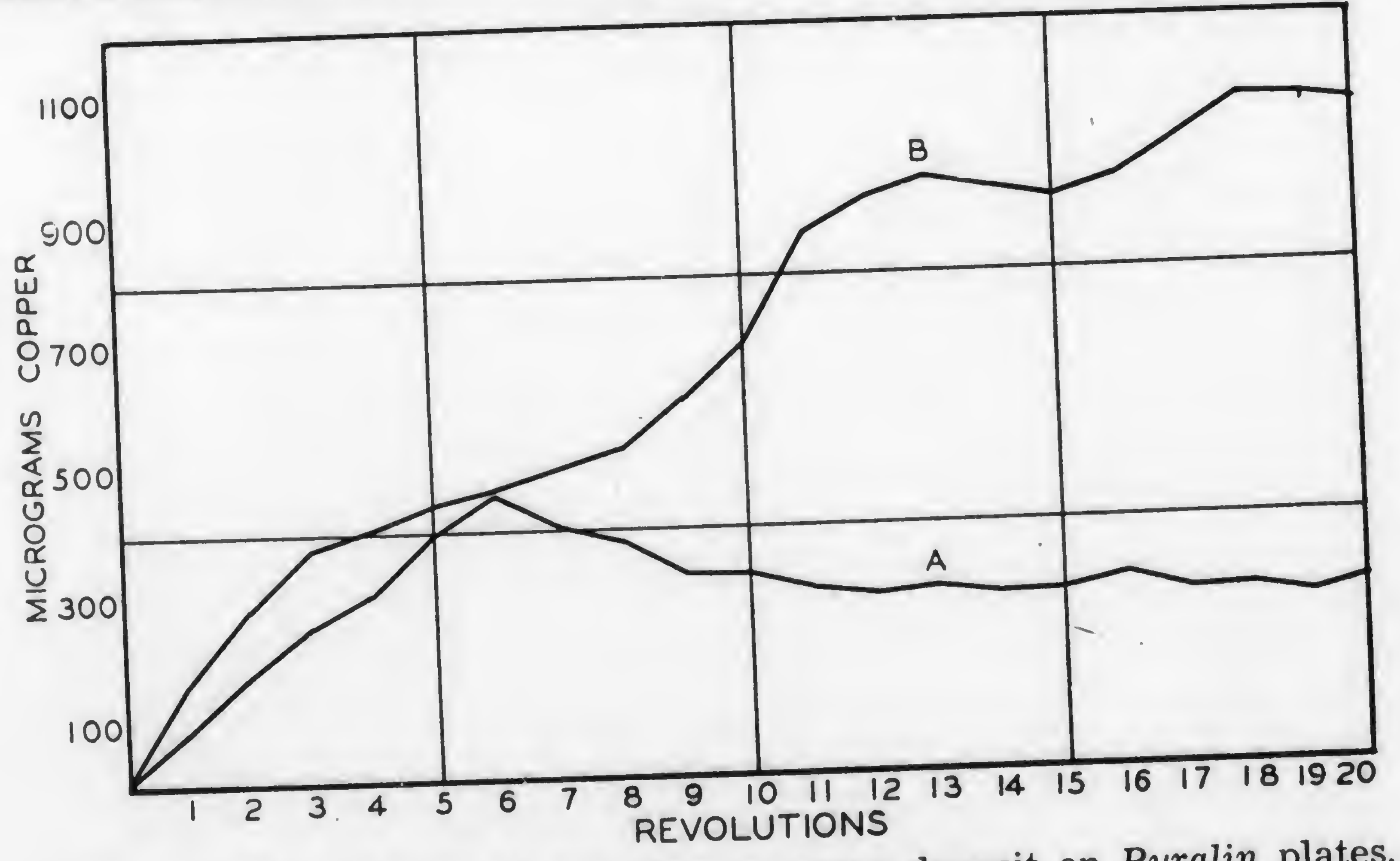


Fig. 8.—Effect of time of spraying on spray deposit on *Pyralin* plates. Line A represents the deposit of BORDOW, discontinuous type deposit; line B, bordeaux, continuous or film-type deposit.

The process of deposit is also shown in figure 9, in which a series of photomicrographs of the deposits presented in figure 8, line A, are shown. The progress of the build-up is clearly visible, as well as the "channeling" and secondary deposit on the cleared areas in the plates receiving 10 and 12 revolutions.

In line B, figure 8, the course of deposit for a material which forms a continuous film-type coverage is shown. In this case a 3-6-100 bordeaux mixture was used. Here there was a rather irregular build-up of deposit which continued as long as the spray was applied.

These two materials are by no means representative of all of the insecticide and fungicide sprays used; rather these results are presented to illustrate possible future studies which may prove of interest. The results certainly indicate that spraying beyond the point of run-off may produce quite variable amounts of deposit.

Retention studies.—The apparatus described in this paper was originally designed to be used for retention studies on insecticide and fungicide deposits, and has been used mainly for this type of investigations (9 and 14). A description of the method of operation and the presentation of typical results may be of interest.

Pyralin plates, sprayed with the desired concentration of insecticide or fungicide as described in the earlier portion of this paper, are allowed to dry and are then sprayed with measured amounts of water delivered through the atomizer nozzle. The table upon which the plates are fastened is, or course, rotated during this process, as in the spraying procedure. The plates are allowed to dry, some are removed for analysis, and the remainder subjected to washing. Usually three applications of water have been made, 500, 1500, and 3500 ml of distilled water being used to wash the plates. It is possible by the use of a suitable collection box to measure the amount of water striking each plate. In general, the deposits of most of the less adhesive materials are reduced to roughly one-third after washing with 3500 ml of distilled water.

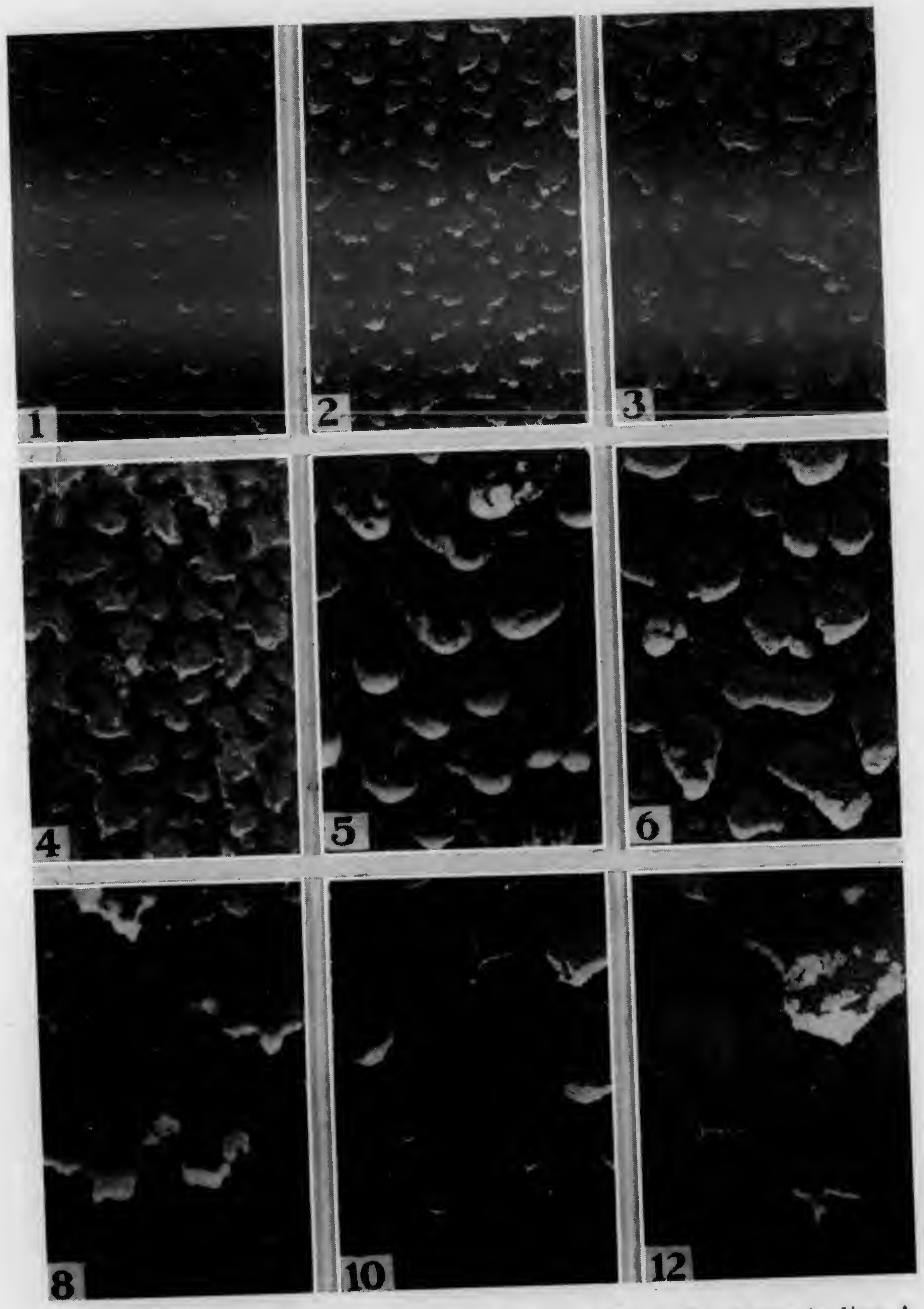


Fig. 9.—Photomicrographs of spray deposits, BORDOW, shown in line A, Fig. 8. The numeral in the corner of each photograph indicates the number of revolutions during which the spray was applied. Note increase in droplet size and ultimate run-off.

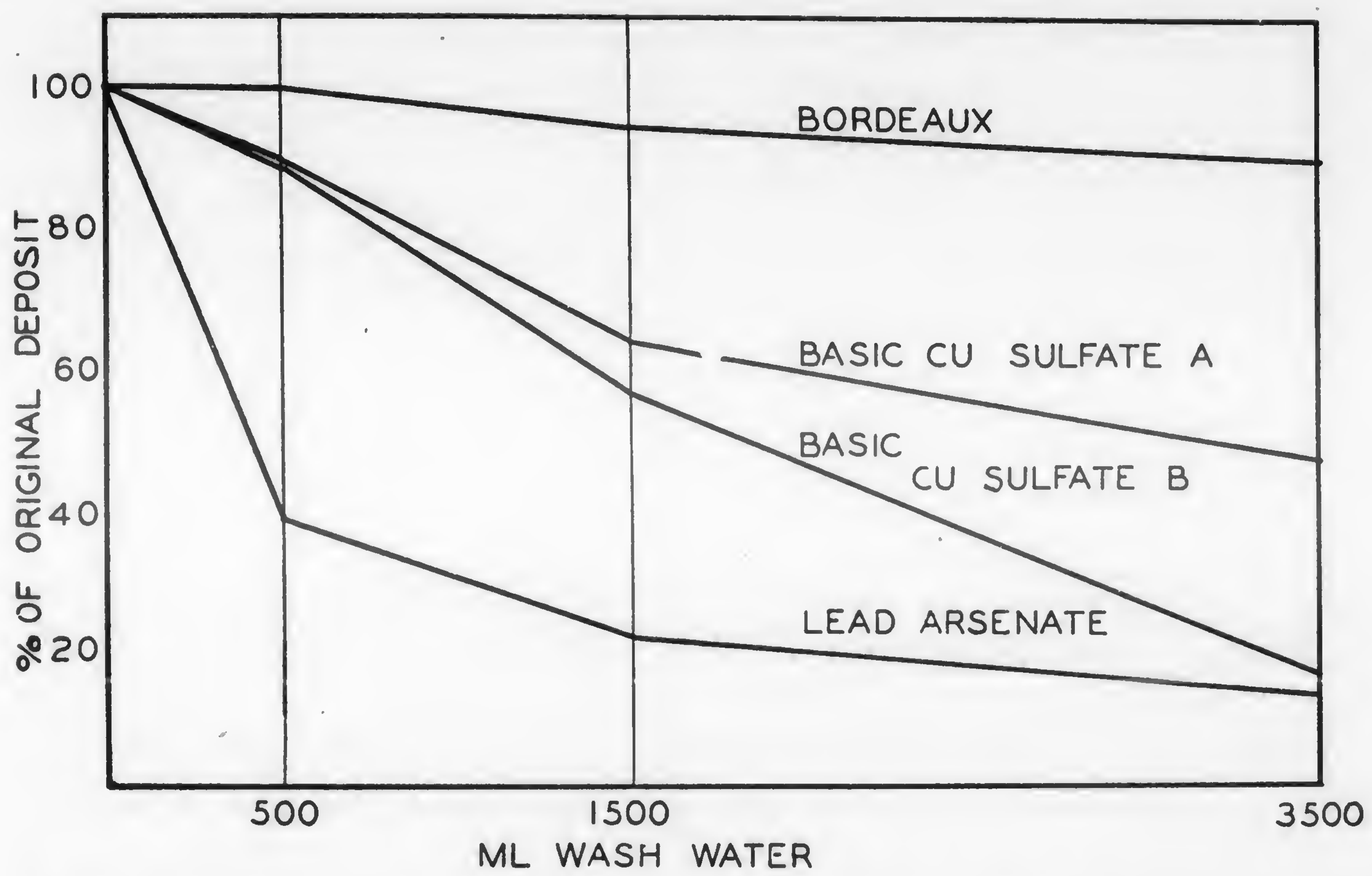


Fig. 10.—Relation between retention of various types of spray deposit and amount of washing.

Typical results for four materials are given in figure 10. It will be noted that in comparison with the other materials tested, bordeaux mixture exhibited a high degree of retention. The two commercial basic copper sulfates were retained to a less extent, and lead arsenate least of all.

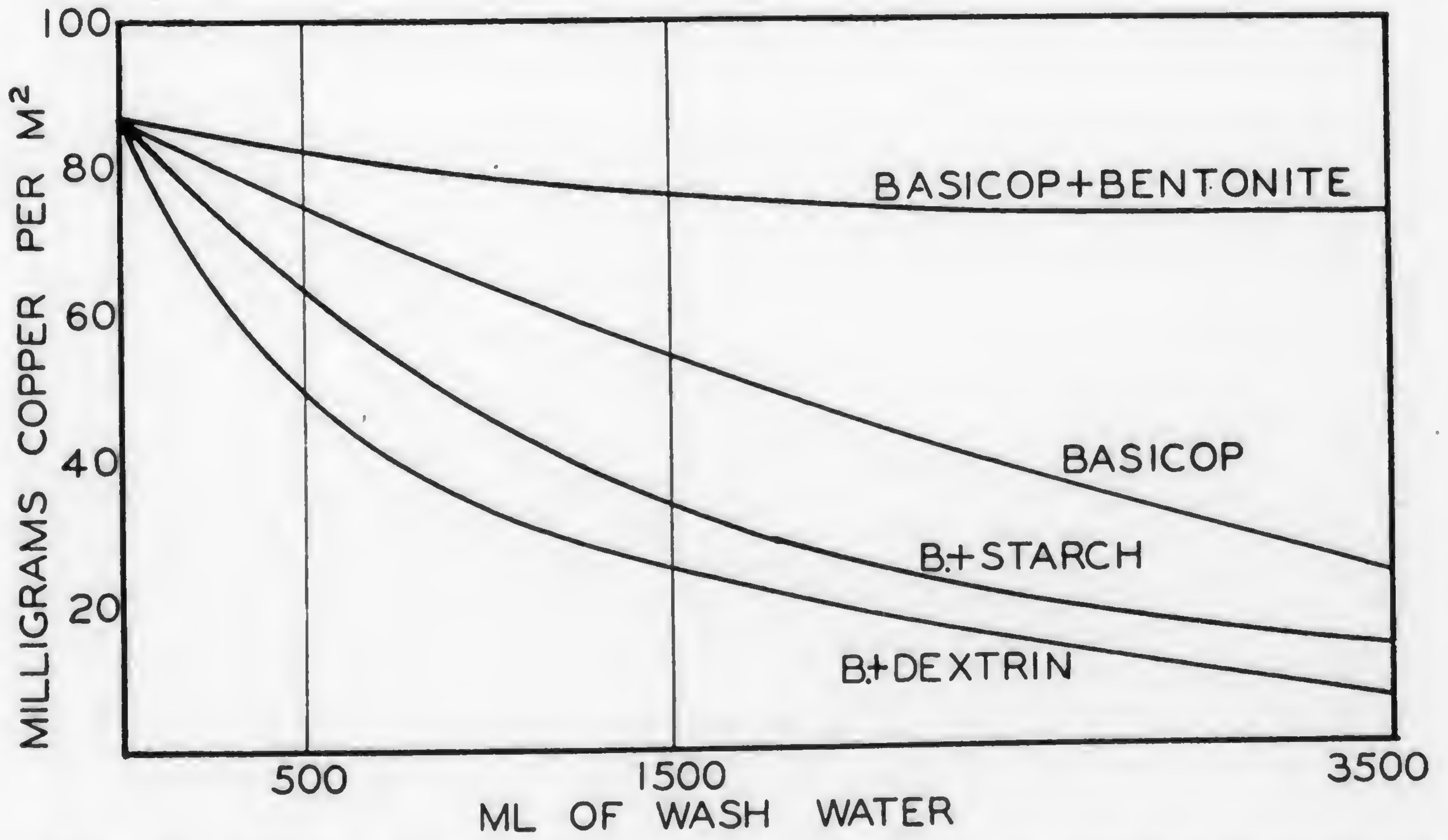


Fig. 11.—Typical results from a study of retention using a commercial copper fungicide with and without adhesive agents.

This method of testing is also well adapted to the study of spray adjuvants, particularly adhesive agents. Figure 11 shows the results of a typical retention study, in which a commercial copper fungicide, BASICOP, was combined with three different adhesives. The differences between the fungicide alone and when used in combination with bentonite, starch, and dextrin are obvious. Comparative studies of a similar nature on several insecticides and fungicides are under way, using a large number of adhesives. Results of this work will be published in the near future.

BIBLIOGRAPHY

(1) Borden, A. D. and G. S. Hensill. 1934. A method of studying comparative oil deposits of proprietary oil emulsions. *Jour. Econ. Ent.* **27**: 834-841.

(2) Dawsey, L. H., A. W. Cressman, and J. Hiley. 1937. The relative quantities of oil deposited upon paraffin-coated plates and upon plant foliage by oil sprays. *Jour. Agr. Res.* **54**:387-398.

(3) Frear, D. E. H. and H. N. Worthley. 1937. Deposition and retention of sprays on apples. *Pa. Agr. Expt. Sta. Bul.* **344**.

(4) Frear, D. E. H. and H. N. Worthley. 1940. Deposition and retention of sprays on apples. II. *Pa. Agr. Expt. Sta. Bul.* **400**.

(5) Horsfall, J. G., J. W. Heuberger, E. G. Sharvelle, and J. M. Hamilton. 1940. A design for laboratory assay of fungicides. *Phytopath.* **30**: 545-563.

(6) Martin, H. 1932. The laboratory examination of fungicidal dusts and sprays. *Ann. Appl. Biol.* **19**: 263-271.

(7) McCallan, S. E. A. and F. Wilcoxon. 1940. An analysis of factors causing variations in spore germination tests of fungicides. II. Methods of Spraying. *Contrib. Boyce Thompson Inst.* **11**: 309-324.

(8) McCallan, S. E. A., Chairman, 1943. Definitions of fungicide terms. *Phytopath.* **33**: 624-626.

(9) Miller, H. J. 1943. A comparison of laboratory and field methods for evaluating retention and protective value of certain copper fungicides. *Phytopath.* **33**: 899-909.

(10) Moore, W. 1921. Spreading and adherence of arsenical sprays. *Minn. Agr. Expt. Sta. Tech. Bul.* **2**.

(11) Newcomer, E. J. 1926. Laboratory experiments with arsenicals in the control of the codling moth. *Jour. Agr. Res.* **33**: 317-330.

(12) Swingle, M. C., A. M. Phillips, and J. B. Gahan. 1941. Laboratory testing of natural and synthetic organic substances as insecticides. *Jour. Econ. Ent.* **34**: 95-99.

(13) Upholt, W. M. and W. M. Hoskins. 1940. Factors concerned in the deposit of sprays. *Jour. Econ. Ent.* **33**: 102-107.

(14) Worthley, H. N. and D. E. H. Frear. 1942. Deposition of lead from lead arsenate spray mixtures and its retention on pyralin plates and apple leaves and fruits. *Jour. Econ. Ent.* **35**: 205-210.

Reprinted from INDUSTRIAL AND ENGINEERING CHEMISTRY, V
Copyright 1944 by the American Chemical Society and reprinted by perm

EFFECT OF
WASTE SULFITE LIQU
Aggregation of S

R. B. ALDERFER, Soil Conservation Service,
U. S. Department of Agriculture, State College, Pa.

M.
The P

ONE of the most important methods for producing wood pulp for paper manufacture involves the digestion of wood chips by a sulfite solution. As a result the lignin of the wood is rendered soluble and leaves cellulose as a residue. After digestion is completed, the spent liquor, usually referred to as waste sulfite liquor, is a dark colored solution consisting mainly of lignin sulfonate, some carbohydrate materials which were originally present in and dissolved from the wood, and a small amount of ash-forming elements. The utilization of waste sulfite liquor, instead of its discharge into streams, is an involved problem worthy of serious study.

It has been estimated that the annual discharge of waste sulfite liquor in the United States approximates 27,000,000 tons, representing more than 1,500,000 tons of lignin (*6*). These figures are in substantial agreement with more recent estimates submitted by Bollen (*2*). Various investigations leading to the utilization of this material over a long period have not met with any considerable degree of success, although some progress has been made. A series of investigations was therefore undertaken to study the possibilities of this waste material, including its effect on soil structure and plant growth.

In the past the bulkiness of the waste product, together with the presence of certain deleterious components, has constituted an obstacle to its possible use as a soil amendment. Now, however, it is being modified by chemical treatment which eliminates the more deleterious components, and is being concentrated by multiple-effect evaporation to a finished solution (Clarion extract) containing 50% dissolved solids. This material, as prepared by the Castanea Paper Company, was used for the experimental work and approximated 6% of ash, 4% nonfermentable carbohydrates, 5% fermentable carbohydrates, and 35% lignin. The carbohydrate and lignin fractions were determined by the Partansky and Benson methods (*4*).

Although the material contains some calcium, potassium, sulfur and certain other elements essential to plant growth, it would not b
fractions
the st
add

would appear, ther
carbohydrates prese
the results obtained.

These treated soil
mixture applied at
to 6 units of nitrog
while the yields w
symptoms of nitroge
The soil organisms
with the tobacco pla
should be given du
is applied to soils.

Clarion extract w
greenhouse condition
applied, noting the
the aggregation of
was applied at the r
was obtained from p
and had received no
exception of nitroger
alternate years over

When the extract
solids per acre a few
seedlings was obtair
and potassium was sa
an area of 2 square
4.98% nitrogen and
germination failed
supply 5 tons of tota
to a lack of aeration
ing effect on the uppe

Samples taken fro
samples 2 and 3 of

TABLE I.

Sample No.	Treatment[a] Tons/Acre
1	Untreat
2	2.5
3	5
4	Untreat
5	2.5
6	5
7	10

[a] Dissolved soli

to effect optir
probable perme
greater percenta
eters >2 mm.,
readily permeal
bility figure is
for particles gr
arrangement of
whether it is sa
the degree of pe

In order to
of the carbohy
varying quanti
outside the gr
from Novembe
treated soil wa
the same time.
for aggregate a
4, 5, 6, and 7 in

The yield of
the intake of
end of 8 wee
A plot which h
received rott
manure at the ra
of 20 tons and
4-8-12 fertili
mixture at the ra
of 1000 poun
per acre, produc
seedlings whic
over a 2 squa
foot are
amounted to
grams (d
weight) and ha
nitrogen conte
of 1.71% and
potassium conte
of 6.30%.
the other ha
the composit

This method of testing is also well adapted to the study of spray adjuvants, particularly adhesive agents. Figure 11 shows the results of a typical retention study, in which a commercial copper fungicide, BASICOP, was combined with three different adhesives. The differences between the fungicide alone and when used in combination with bentonite, starch, and dextrin are obvious. Comparative studies of a similar nature on several insecticides and fungicides are under way, using a large number of adhesives. Results of this work will be published in the near future.

BIBLIOGRAPHY

(1) Borden, A. D. and G. S. Hensill. 1934. A method of studying comparative oil deposits of proprietary oil emulsions. *Jour. Econ. Ent.* **27**: 834-841.

(2) Dawsey, L. H., A. W. Cressman, and J. Hiley. 1937. The relative quantities of oil deposited upon paraffin-coated plates and upon plant foliage by oil sprays. *Jour. Agr. Res.* **54**:387-398.

(3) Frear, D. E. H. and H. N. Worthley. 1937. Deposition and retention of sprays on apples. *Pa. Agr. Expt. Sta. Bul.* **344**.

(4) Frear, D. E. H. and H. N. Worthley. 1940. Deposition and retention of sprays cn apples. II. *Pa. Agr. Expt. Sta. Bul.* **400**.

(5) Horsfall, J. G., J. W. Heuberger, E. G. Sharvelle, and J. M. Hamilton. 1940. A design for laboratory assay of fungicides. *Phytopath.* **30**: 545-563.

(6) Martin, H. 1932. The laboratory examination of fungicidal dusts and sprays. *Ann. Appl. Biol.* **19**: 263-271.

(7) McCallan, S. E. A. and F. Wilcoxon. 1940. An analysis of factors causing variations in spore germination tests of fungicides. II. Methods of Spraying. *Contrib. Boyce Thompson Inst.* **11**: 309-324.

(8) McCallan, S. E. A., Chairman, 1943. Definitions of fungicide terms. *Phytopath.* **33**: 624-626.

(9) Miller, H. J. 1943. A comparison of laboratory and field methods for evaluating retention and protective value of certain copper fungicides. *Phytopath.* **33**: 899-909.

(10) Moore, W. 1921. Spreading and adherence of arsenical sprays. *Minn. Agr. Expt. Sta. Tech. Bul.* **2**.

(11) Newcomer, E. J. 1926. Laboratory experiments with arsenicals in the control of the codling moth. *Jour. Agr. Res.* **33**: 317-330.

(12) Swingle, M. C., A. M. Phillips, and J. B. Gahan. 1941. Laboratory testing of natural and synthetic organic substances as insecticides. *Jour. Econ. Ent.* **34**: 95-99.

(13) Upholt, W. M. and W. M. Hoskins. 1940. Factors concerned in the deposit of sprays. *Jour. Econ. Ent.* **33**: 102-107.

(14) Worthley, H. N. and D. E. H. Frear. 1942. Deposition of lead from lead arsenate spray mixtures and its retention on pyralin plates and apple leaves and fruits. *Jour. Econ. Ent.* **35**: 205-210.

Reprinted from INDUSTRIAL AND ENGINEERING CHEMISTRY, Vol. 36, Page 272, March, 1944

Copyright 1944 by the American Chemical Society and reprinted by permission of the copyright owner

EFFECT OF WASTE SULFITE LIQUOR ON Aggregation of Soil Particles

R. B. ALDERFER, Soil Conservation Service,
U. S. Department of Agriculture, State College, Pa.

M. F. GRIBBINS AND D. E. HALEY
The Pennsylvania State College, State College, Pa.

ONE of the most important methods for producing wood pulp for paper manufacture involves the digestion of wood chips by a sulfite solution. As a result the lignin of the wood is rendered soluble and leaves cellulose as a residue. After digestion is completed, the spent liquor, usually referred to as waste sulfite liquor, is a dark colored solution consisting mainly of lignin sulfonate, some carbohydrate materials which were originally present in and dissolved from the wood, and a small amount of ash-forming elements. The utilization of waste sulfite liquor, instead of its discharge into streams, is an involved problem worthy of serious study.

It has been estimated that the annual discharge of waste sulfite liquor in the United States approximates 27,000,000 tons, representing more than 1,500,000 tons of lignin (*6*). These figures are in substantial agreement with more recent estimates submitted by Bollen (*2*). Various investigations leading to the utilization of this material over a long period have not met with any considerable degree of success, although some progress has been made. A series of investigations was therefore undertaken to study the possibilities of this waste material, including its effect on soil structure and plant growth.

In the past the bulkiness of the waste product, together with the presence of certain deleterious components, has constituted an obstacle to its possible use as a soil amendment. Now, however, it is being modified by chemical treatment which eliminates the more deleterious components, and is being concentrated by multiple-effect evaporation to a finished solution (Clarion extract) containing 50% dissolved solids. This material, as prepared by the Castanea Paper Company, was used for the experimental work and approximated 6% of ash, 4% nonfermentable carbohydrates, 5% fermentable carbohydrates, and 35% lignin. The carbohydrate and lignin fractions were determined by the Partansky and Benson methods (*4*).

Although the material contains some calcium, potassium, sulfur and certain other elements essential to plant growth, it would not be classed as a fertilizer. Its lignin and carbohydrate fractions, however, offer possibilities as agents for improving the structural properties of soils through bacterial action and add to the humus supply through the formation of bacterial protein-lignin complexes.

EFFECT ON FRESHLY PLOWED SOILS

The first series of experiments included the addition of Clarion extract to freshly plowed soils of the Hagerstown series. The solution was diluted and enough of the material was used per acre to supply 5 tons of total solids. When thoroughly dry, the soil was cultivated and planted to tobacco. The beneficial effect of these treatments on the aggregation of soil particles was marked and was apparent long after the crop was harvested.

Through the bacterial decomposition of sugar in the soil, Peele (*5*) was able to show an increase in soil aggregation. It would appear, therefore, that, in addition to the lignin, the carbohydrates present in the liquor must have played a part in the results obtained.

These treated soils were supplemented with a 3–8–12 fertilizer mixture applied at the rate of 1000 pounds per acre. From 4 to 6 units of nitrogen, however, should have been used; for while the yields were satisfactory, the plants showed some symptoms of nitrogen deficiency as they approached maturity. The soil organisms which effect decomposition were competing with the tobacco plants for this element. This biological effect should be given due consideration when this waste material is applied to soils.

GREENHOUSE TESTS

Clarion extract was then used on tobacco seedbeds under greenhouse conditions and to increase the quantity of fertilizer applied, noting the ultimate effect on seedling growth and on the aggregation of soil particles. A 4–8–12 fertilizer mixture was applied at the rate of 1500 pounds per acre. The soil used was obtained from plot 2, tier III, of the Jordan fertility plots, and had received no fertilizer treatment for 58 years with the exception of nitrogen as dried blood which had been applied in alternate years over this period.

When the extract was applied at the rate of 2.5 tons of total solids per acre a few days before seeding, a good yield of tobacco seedlings was obtained, and the absorption of both nitrogen and potassium was satisfactory. The dry weight of seedlings over an area of 2 square feet amounted to 33 grams and contained 4.98% nitrogen and 8.31% potassium (*3*). On the other hand, germination failed when sufficient material was applied to supply 5 tons of total solids per acre. This was due principally to a lack of aeration because of a temporary physical cementing effect on the upper soil layer by the undecomposed material.

Samples taken from these soils after 8 weeks, as indicated by samples 2 and 3 of Table I, showed a marked aggregation of soil particles. The degree to which the particles of the original untreated field soil were aggregated into good tilth-producing crumbs, following the addition of the extract, was determined by structural stability and permeability measurements (*1*). Stability reflects the degree to which the clay, silt, and fine sand have been aggregated into larger water-stable granules. A high stability index indicates that much of the silt and clay (two soil particle sizes present in high quantity in the Hagerstown silty clay loam soil) have been aggregated into large, stable granules or crumbs. The latter tend to produce a good tilth, whereas the soil in a dispersed condition results in poor structure or tilth. The stability index is the sum of the positive differences between the percentage of soil existing in the various larger size classes (>0.02 mm.) by aggregate and mechanical analysis. Probable permeability is represented by the percentage of the soil which consists of either aggregates or stones large enough

Table I. Relation of Waste Sulfite Liquor Treatment to Soil Structure

Sample No.	Treatment[a], Tons/Acre	Stability Index	Probable Permeability	Analysis	Gravel (>2 Mm.), %	Coarse Sand (2–1 Mm.), %	Medium Sand (1–0.2 Mm.), %	Fine Sand (0.2–0.02 Mm.), %	Silt (0.02–0.002 Mm.), %	Clay (<0.002 Mm.), %
				Sampled after Plant Harvest						
1	Untreated	58.5	30.1	Aggregate	8.54	5.07	16.45	60.89	8.71	0.34
				Mechanical	6.43	1.75	3.69	20.54	45.62	21.97
2	2.5	62.8	58.1	Aggregate	23.89	8.01	26.15	37.11	4.63	0.21
				Mechanical	4.58	1.81	3.95	22.07	45.62	21.97
3	5	64.9	64.6	Aggregate	23.20	11.78	29.64	32.66	2.36	0.36
				Mechanical	3.73	2.43	3.89	22.36	45.62	21.97
				Sampled in Early Spring						
4	Untreated	50.9	20.5	Aggregate	4.92	4.32	11.24	62.80	15.82	0.90
				Mechanical	2.61	2.05	3.36	24.39	45.62	21.97
5	2.5	55.8	29.3	Aggregate	6.64	4.45	18.18	58.95	11.38	0.20
				Mechanical	3.47	2.00	3.35	23.59	45.62	21.97
6	5	61.2	49.1	Aggregate	8.81	8.81	31.46	44.52	6.22	0.18
				Mechanical	3.72	2.59	4.56	21.54	45.62	21.97
7	10	64.3	69.1	Aggregate	17.06	18.06	34.00	27.60	3.09	0.19
				Mechanical	3.42	1.36	3.11	24.52	45.62	21.97

[a] Dissolved solids in the form of concentrated extract.

to effect optimum moisture-permeability relations. A high probable permeability figure represents a condition in which a greater percentage of the soil consists of particles having diameters >2 mm., which if arranged properly will render the soil readily permeable to air and moisture. The probable permeability figure is the sum of the aggregate analysis percentages for particles greater than 0.2 mm. Soil structure refers to the arrangement of soil particles and may be characterized as to whether it is satisfactory or unsatisfactory for plant growth by the degree of permeability of the soil to air and moisture.

In order to ensure a more or less thorough decomposition of the carbohydrates present, soils were intimately mixed with varying quantities of the Clarion extract and placed in heaps outside the greenhouse. They were exposed to the weather from November, 1941, to March, 1942. A sample of the untreated soil was exposed to the same weather conditions for the same time. At the end of the period samples were taken for aggregate analysis; the results are represented by samples 4, 5, 6, and 7 in Table I.

The yield of seedlings produced on these treated soils and the intake of nitrogen and potassium were obtained at the end of 8 weeks. A plot which had received rotted manure at the rate of 20 tons and a 4-8-12 fertilizer mixture at the rate of 1000 pounds per acre, produced seedlings which, over a 2 square foot area, amounted to 22 grams (dry weight) and had a nitrogen content of 1.71% and a potassium content of 6.30%. On the other hand, the composited soil receiving the extract at the rate of 10 tons of total solids per acre and a similar fertilizer treatment (represented by sample 7, Table I) produced 24 grams (dry weight) of seedlings having a nitrogen content of 3.84% and a potassium content of 7.27% (*3*).

Table II. Effect of Waste Sulfite Liquor on Soil Structure as Influenced by Manure and Fertilizer Treatment

	Treatment per Acre											
Sample No.	W.S.L.[a], tons	Manure, tons	Fertilizer (4–8–12), lb.	Stability Index	Probable Permeability	Analysis	Gravel, %	Coarse Sand, %	Medium Sand, %	Fine Sand, %	Silt, %	Clay, %
1–14	0	0	0	52.24	18.63	Aggregate	5.37	3.87	9.39	62.49	17.85	1.03
						Mechanical	3.82	1.37	3.06	20.63	48.70	22.42
2–15	2.5	0	0	60.23	32.33	Aggregate	6.96	5.08	20.29	56.78	10.17	0.39
						Mechanical	2.92	1.29	3.14	21.53	48.70	22.42
5–18	5.0	0	0	61.61	42.49	Aggregate	10.02	7.77	24.70	48.00	8.94	0.57
						Mechanical	3.46	1.67	3.24	20.51	48.70	22.42
8–21	0	10	0	48.59	20.44	Aggregate	7.01	4.12	9.31	57.03	21.56	0.98
						Mechanical	3.19	1.74	3.57	20.38	48.70	22.42
9–22	2.5	10	0	59.03	37.14	Aggregate	8.79	5.70	22.65	50.59	11.61	0.33
						Mechanical	2.79	1.28	3.05	21.58	48.70	22.42
10–23	5.0	10	0	62.07	45.35	Aggregate	10.59	7.89	26.87	45.60	8.61	0.43
						Mechanical	3.52	1.93	3.84	19.59	48.70	22.42
11–24	0	10	1000	51.47	20.42	Aggregate	6.42	4.24	9.76	59.93	18.80	0.86
						Mechanical	3.06	1.35	3.15	21.32	48.70	22.42
3–16	2.5	0	1000	53.19	25.73	Aggregate	5.58	4.63	15.52	56.34	16.86	1.07
						Mechanical	3.01	1.27	2.94	21.66	48.70	22.42
6–19	5.0	0	1000	64.07	50.81	Aggregate	13.65	8.55	28.61	42.14	6.64	0.41
						Mechanical	3.92	1.11	3.00	20.76	48.70	22.42
12–25	2.5	10	1000	59.82	38.12	Aggregate	8.98	6.07	23.07	50.58	10.75	0.54
						Mechanical	3.13	1.34	2.55	21.86	48.70	22.42
13–26	5.0	10	1000	63.17	47.06	Aggregate	14.12	7.60	25.34	44.99	7.08	0.87
						Mechanical	4.31	1.26	2.87	20.44	48.70	22.42

[a] Dissolved solids in the form of the concentrated extract.

RESULTS WITH MANURE AND FERTILIZER

An effort was next made to ascertain the effect of Clarion extract on soil aggregation when used alone, with rotted manure, with fertilizer, or with both. Soil was obtained from a different part of the plot previously mentioned. The experiment was conducted under greenhouse conditions over a 2-month period. The treatments and results are given in Table II. The mechanical and aggregate analyses were conducted on composite samples taken from duplicate plots of the same treatment. The results show a marked effect of the extract on the aggregation of soil particles, which was intensified by supplemental fertilizer treatments.

These studies indicate that an excellent soil structure may be obtained through the judicious use of the sulfite liquor. There is a marked increase in probable permeability which, in turn, is brought about by the considerable increase in the number of water-stable granules produced as shown by aggregate analysis.

If this material is to be used as a soil amendment, the quantity to apply must be watched carefully, and intimate mixing with the soil particles must be insured. A few weeks at least should elapse before crops are seeded on soil so treated, in order to provide a suitable interval for soil organisms to decay the less resistant materials. To ensure more rapid and thorough decomposition of these materials, the addition of an ample quantity of a well balanced fertilizer mixture should precede the treatment. This mixture should carry from 40 to 60 pounds per acre of readily available nitrogen when the equivalent of 5 tons of dissolved solids are used. Such an application should prevent serious competition between the soil organisms and

crop plants for the mineral nutrients present. It should also contribute to the formation of humus, since most of the humus found in soils results from a combination of lignin with bacterial proteins.

ACKNOWLEDGMENT

The work reported in this paper was made possible through a fellowship established at The Pennsylvania State College by the Castanea Paper Company, of Johnsonburg, Pa.

LITERATURE CITED

(1) Alderfer, R. B., and Merkle, F. G., *Soil Sci.*, 51, 201 (1941).

(2) Bollen, W. B., *Pacific Pulp & Paper Ind.*, 16, 30 (1942).

(3) Gribbins, M. F., Ph.D. thesis, Pa. State College, 1942.

(4) Partansky, A. M., and Benson, H. K., *Paper Trade J.*, **101**, 29–35 (1936).

(5) Peele, T. C., *J. Am. Soc. Agron.*, 32, 204 (1940).

(6) Phillips, M., *et al.*, *J. Agr. Research*, 53, 209 (1936).

Journal Series Paper 1156, Pennsylvania Agricultural Experiment Station.

Printed in U. S. A.

Reprinted from INDUSTRIAL AND ENGINEERING CHEMISTRY, V
Copyright 1944 by the American Chemical Society and reprinted by perm

FANWEED SEED OIL

Potential Substitute for Rapeseec

J. R. CLOPTON[1] AND
H. O. TRIEBOLD
The Pennsylvania State College,
State College, Pa.

**Fanweed (*Thlaspi arvense*) seeds contain
properties are similar to those of rapeseed
terized by a high content of erucic acid. Fa
higher in linoleic acid than those of rapeseed
nary temperatures are similar, and their
are comparable. This property suggests t
place of rapeseed oil as a lubricant constitue**

DURING 1940 the United States imported 12,919,000 pounds of rapeseed oil (*8*). Of that quantity 11,595,000 pounds or nearly 90% came from Japan and the Japanese-held territory of Kwantung. Argentina supplied the remaining 10%. Since importations from the East are now impossible, it would be highly desirable if an oil could be produced in the United States for the same uses as rapeseed. Such a possibility
therefore
in cor
F

It matures early (a
season possible. Th
inches and can be ha
ment. A yield of 5
season would compare

PHYSICAL A

were determined on
illation. Using these
, it was possible to
on qualitatively and

Journal Series Paper 1187, Pennsylvania Agricultural Experiment Station.

(7) Schuette, H. A., and Roberts, W. L., Ind. Eng. Chem., Anal. Ed., 4, 257-9 (1932).
(8) U. S. Tariff Comm., "Fats, Oils, and Oil-Bearing Materials in the United States", 1941.
(9) Whitmore, F. C., and Lux, A. R., *J. Am. Chem. Soc.*, 54, 3448-54 (1932).

Printed in U. S. A.

responding valu
refraction, iodin
tion point for fa
rated than rapes
weed seed oil is
is rapeseed oil.

To compare t
oils, it was nece
of individual ac
The oil was sapo
the soaps were
matter. The so
the fatty acids
ether extracts
free of mineral
sulfate. The et
by distillation u

Methyl esters
acids 3 hours wi
and a little co
removed; the
volume of wate
to remove any
bined with the
sulfate. The dr
of the ether und

The mixed m
jected to fractio
tionating colum
(*9*), equipped w
and packed with
were electrically
by water-coole
followed by dete
fraction as it wa
refractive indice

TABLE I. P
F

Specific gravity, d_1^1
Refractive index, n
Viscosity
Saybolt seconds
20.0° C.
37.8° C.
98.9° C.
Kinematic cent
20.0° C.
37.8° C.
98.9° C.
Viscosity index
Iodine value
Saponification valu
Free fatty acid valu
Ester value
Unsaponifiable ma
Soluble fatty acids,
Insoluble fatty acid
Reichert Meissl va
Polenske value
Hydroxyl number
Acetyl value
Insoluble fatty acid
Iodine value
Acid value
Mean molecular
Melting point, °
Solidification poi

Reprinted from INDUSTRIAL AND ENGINEERING CHEMISTRY, Vol. 36, Page 218, March, 1944

Copyright 1944 by the American Chemical Society and reprinted by permission of the copyright owner

FANWEED SEED OIL

Potential Substitute for Rapeseed Oil

J. R. CLOPTON[1] **AND**
H. O. TRIEBOLD
The Pennsylvania State College,
State College, Pa.

Fanweed (*Thlaspi arvense*) seeds contain 33 to 35% oil. The composition and properties are similar to those of rapeseed oil. Glycerides of both oils are characterized by a high content of erucic acid. Fanweed seed oil glycerides are somewhat higher in linoleic acid than those of rapeseed oil. Viscosities of the two oils at ordinary temperatures are similar, and their changes in viscosity with temperature are comparable. This property suggests that fanweed seed oil could be used in place of rapeseed oil as a lubricant constituent and for other industrial purposes.

DURING 1940 the United States imported 12,919,000 pounds of rapeseed oil (*8*). Of that quantity 11,595,000 pounds or nearly 90% came from Japan and the Japanese-held territory of Kwantung. Argentina supplied the remaining 10%. Since importations from the East are now impossible, it would be highly desirable if an oil could be produced in the United States for the same uses as rapeseed. Such a possibility appeared to exist in fanweed seed oil; a study was therefore made of the composition and properties of this oil in comparison with those of rapeseed oil.

Fanweed grows abundantly in most of the northwestern and north central states, as well as in southwestern Canada. In the western United States it is commonly called fanweed, but is known also as Frenchweed or pennycress. Its botanical name is *Thlaspi arvense*, and it is a member of the Cruciferae, to which rape and members of the mustard family belong. Therefore, it was to be expected that fanweed seed oil would be similar in properties to rape and mustard oils.

Fanweed seeds contain 33 to 35% oil by weight. Test plantings at the Montana State College show that on irrigated land 1500 pounds of seed per acre can be obtained. The plant is hardy and grows well without irrigation, except on very dry land. It matures early (around July 1) making two crops in one season possible. The fanweed attains a height of 12 to 18 inches and can be harvested easily with the usual farm equipment. A yield of 500 to 1000 pounds of oil per acre in one season would compare favorably with other oil-producing crops.

PHYSICAL AND CHEMICAL CHARACTERISTICS

The seeds, freed from foreign matter, were ground to pass a 20-mesh sieve. The oil was extracted with petroleum ether (boiling at 30–60° C.) in a modified Soxhlet extractor. Nearly all of the solvent was removed from the oil by distillation. The last traces were removed in vacuo while passing a small stream of nitrogen gas through the oil. The extracted oil was light green and had a mild, pleasant odor and flavor. During storage the color, due to chlorophyll, gradually disappeared, and the oil assumed a golden yellow color.

The physical and chemical characteristics of freshly extracted fanweed seed oil are reported in Table I. The values obtained were determined by the commonly accepted methods for fat analysis (*1, 7*). For comparison, the corresponding characteristics for rapeseed oil, taken from the literature (*2, 4, 5*), are given in Table I. The data show that in most instances the values obtained on fanweed seed oil compare favorably with the corresponding values for rapeseed. The somewhat higher index of refraction, iodine number, and density, and the lower solidification point for fanweed seed oil indicate that it is more unsaturated than rapeseed oil. These characteristics suggest that fanweed seed oil is more closely related to a semidrying oil than is rapeseed oil.

---

[1] Present address, Armour Research Foundation, Chicago, Ill.

COMPONENT FATTY ACIDS

To compare the compositions of fanweed seed and rapeseed oils, it was necessary to identify and determine the percentage of individual acids comprising the glycerides of the two oils. The oil was saponified with alcoholic potassium hydroxide, and the soaps were washed with ether to remove unsaponifiable matter. The soaps were acidified with sulfuric acid to liberate the fatty acids which were then extracted with ether. The ether extracts were combined and washed with water until free of mineral acid and then dried with anhydrous sodium sulfate. The ether was removed from the mixed fatty acids by distillation under reduced pressure.

Methyl esters were prepared by refluxing the mixed fatty acids 3 hours with four times their weight of absolute methanol and a little concentrated sulfuric acid. The ester layer was removed; the alcohol layer was diluted with three times its volume of water and extracted with several portions of ether to remove any dissolved esters. The ether extracts were combined with the ester layer and dried over anhydrous sodium sulfate. The dry methyl esters were then obtained by removal of the ether under reduced pressure.

The mixed methyl esters of the fatty acids were then subjected to fractional distillation at 1 mm. pressure with a fractionating column of the type described by Whitmore and Lux (*9*), equipped with a total-reflux partial-takeoff distilling head and packed with single-turn glass helices. The bath and jacket were electrically heated, and the temperature was controlled by water-cooled rheostats. The course of distillation was followed by determining the weight and refractive index of each fraction as it was obtained and by plotting a curve showing the refractive indices *vs.* total grams distilled.

Table I. Physical and Chemical Characteristics of Fanweed Seed and Rapeseed Oils

	Fanweed Seed Oil	Rapeseed Oil
Specific gravity, d_{15}^{15}	0.9168	0.913–0.917
Refractive index, n_D^{45}	1.4652	1.4629–1.4639
Viscosity		
Saybolt seconds		
20.0° C.	426.7	432.0
37.8° C.	210.2	213.5
98.9° C.	58.0	54.0
Kinematic centistokes		
20.0° C.	92.56	...
37.8° C.	45.40	...
98.9° C.	9.64	...
Viscosity index	157	...
Iodine value	117.3	98–106
Saponification value	177.8	168–180
Free fatty acid value	0.8	0.36–1.0
Ester value	177.0	...
Unsaponifiable matter, %	1.34	0.8–1.5
Soluble fatty acids, %	0.15	...
Insoluble fatty acids, %	95.7	94.5–96.3
Reichert Meissl value	0.25	0.0–0.79
Polenske value	0.00	...
Hydroxyl number	8.8	...
Acetyl value	8.3	14.7
Insoluble fatty acids		
Iodine value	119.3	100–106
Acid value	186.2	...
Mean molecular weight	301.3	...
Melting point, ° C.	16–18	18.5–20.0
Solidification point, ° C.	10–12	11.7–13.6

Iodine values and neutral equivalents were determined on each of the fractions obtained during distillation. Using these values together with the fraction weight, it was possible to estimate the esters present in each fraction qualitatively and quantitatively and, subsequently, the fatty acid composition of the oil as reported in Table II.

It is significant that the percentage of erucic acid is very high in the glycerides of both fanweed seed and rapeseed oils. This high erucic acid content is responsible for the peculiar characteristics of these oils and also their industrial importance.

Fanweed seed oil glycerides have a higher linoleic acid and a lower oleic acid content than those of rapeseed oil. The effect of the increased linoleic acid is more pronounced than the decrease in oleic acid content, as evidenced by the higher iodine number and lower solidification point of fanweed seed oil.

Table II. Component Fatty Acids of Fanweed Seed and Rapeseed Oils, in Per Cent

Fatty Acid	Fanweed Oil	Rapeseed Oil	Fatty Acid	Fanweed Oil	Rapeseed Oil
Myristic	Trace	Trace	Linolenic	0.5	1.0
Palmitic	1.5	5.0	Erucic	49.0	52.0
Oleic	12.5	17.0	Lignoceric	3.5	1.0
Linoleic	33.0	24.0			

Except for the differences exhibited in the percentages of linoleic and oleic acid, the component fatty acids contents of fanweed seed and rapeseed oils are similar. This suggests that the two oils should show comparable physical and chemical characteristics.

POSSIBLE INDUSTRIAL IMPORTANCE

The relatively high viscosity of rapeseed oil, especially after it is blown, has led to its use as a lubricant constituent. The viscosity of fanweed seed oil is almost the same as that of rapeseed oil; if anything, it maintains a slightly higher viscosity at elevated temperatures than does rapeseed oil. In view of this fact and of the similarity in composition of the two oils, it appears that fanweed seed oil could be substituted for rapeseed oil as a lubricant constituent.

There is good reason to believe that fanweed seed oil could serve as a substitute for rapeseed oil in many other industrial processes such as the manufacture of artgum, rubber substitutes, and materials used in compounding rubber. By proper treatment and blending of fanweed seed oil with drying oils, it might well be used in paints and varnishes and in other products involving drying oils.

In Russia (*3, 6*) an edible oil has been prepared from fanweed seed grown in Siberia. It seems likely that fanweed seed oil could, with proper purification, be used as a shortening or salad oil along with corn, cottonseed, peanut, and other vegetable oils.

In view of the present shortage of oils in this country the possibilities of mass production and processing of fanweed seed should be investigated. Further work is in progress to determine possible industrial uses of fanweed seed oil.

LITERATURE CITED

(1) Assoc. of Official Agr. Chem., Official and Tentative Methods of Analysis, 1940.
(2) Hilditch, T. P., "Industrial Chemistry of Fats and Waxes", New York, D. Van Nostrand Co., 1941.
(3) Ivanov, S., and Troitzkiĭ, N., *Maslobočno Zhirovoe Delo*, 1, 30–1 (1928).
(4) Jamieson, G. S., "Vegetable Fats and Oils", A. C. S. Monograph Series 58, New York, Chemical Catalog Co., 1932.
(5) Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats and Waxes", 6th ed., London, MacMillan Co., 1921.
(6) Milashevskiĭ, V., *Maslobočno Zhirovoe Delo*, 10, 46–7 (1932).
(7) Schuette, H. A., and Roberts, W. L., Ind. Eng. Chem., Anal. Ed., 4, 257–9 (1932).
(8) U. S. Tariff Comm., "Fats, Oils, and Oil-Bearing Materials in the United States", 1941.
(9) Whitmore, F. C., and Lux, A. R., *J. Am. Chem. Soc.*, 54, 3448–54 (1932).

Journal Series Paper 1187, Pennsylvania Agricultural Experiment Station.

Printed in U. S. A.

PERFORMANCE OF NEW SPRAY EQUIPMENT

H. J. Miller, D. E. H. Frear, H. L. King, and F. N. Fagan,
Pennsylvania State College.

Fruit growers are now trying to carry out the normal spray program with more expensive labor and less of it. This has created an acute demand for some kind of spray machinery which will eliminate some of the costly labor involved in getting adequate control of codling moth and scab. Several of you are already using speed sprayers as a means of reducing labor costs and speeding up coverage.

The speed sprayer was introduced into Delaware in 1941, and its performance has been studied by the experiment station(1,2). Their results indicate that the tops of the trees did not receive as much spray as the bottom. The New Jersey experiment station (3) has reported that the coverage with the speed sprayer was about as good in the tops as with the standard type high pressure equipment using brooms. The bottom received more than the tops with both types, indicating that poorer protection against codling moth was obtained in the tops. This is unfortunate since coverage in the tops has been the chief difficulty with our regular type sprayers using guns and brooms.

In the spring of 1943, the college started spraying their orchard with a speed sprayer under the direction of Prof. Fagan. Since the question of adequate coverage in the tops is so important, it seems advisable to compare the performance of this machine with the regular equipment. The trees were quite large since they were 27 years old. The Stayman were approximately 24 feet high, while the McIntosh were 30. Thus a real test of coverage was offered by these trees.

The 500-gallon speed sprayer was hauled through the orchard by a tractor and operated by the driver of the tractor. A truck mounted supply tank was used to refill the sprayer in the orchard. Agitation power and refilling pump for the supply tank was operated by a power take-off from the supply truck engine. The supply tank operator mixed the spray material as the tank was being filled with water at the loading station. These two men operating truck and sprayer are classed as a spray unit. The high pressure sprayer was hauled through the orchard by a track tractor. The spraying was done by one man with an eight nozzle spray broom operated by hand from the spray tower supplemented in the cover sprays by a man on the ground with a gun inside the tree. The high pressure outfit was not serviced in the orchard by supply tank and the two men returned to the filling station for refilling and spray mixing.

The speed sprayer unit averaged in the first four sprays 2.36 times as fast as was the spraying done by the two men operating the 35-gallon high pressure pump with a 400-gallon tank. In the four cover sprays, the speed sprayer unit averaged 2.48 times as fast with a two-man crew, as did the high pressure sprayer unit with a three-man crew.

Samples for lead analysis were taken immediately after each cover spray from both tops and bottoms of each of six trees in each plot. These data were averaged and expressed as milligrams of lead per square meter with both surfaces considered on the leaves. The results of these studies which are summarized in table I show that the high pressure equipment gave approximately equal deposits in top and bottom indicating a fairly uniform coverage. However, the speed sprayer was somewhat disappointing in this respect. This machine put more material on the bottoms and considerably less on the tops. Scab control was better in the tops with the high pressure sprayer. There was no codling moth infestation in this orchard, so a comparison of control of this insect was not possible.

The lead deposit in different parts of the other branches of the upper third of the tree did not seem to vary significantly on different sides with either type of equipment. Packing house grading records on the McIntosh gave an average of 9.6 percent scab with the speed sprayer against 4.8 percent on the high pressure sprayed rows. The speed sprayer applied slightly more material as indicated by an average of 17.6 gallons per tree against 15.3 for the high pressure.

One approach to this problem of better coverage with less labor has been developed at the Delaware station (2). Standard type gun nozzles are mounted on a mast attached to the back of a regular high pressure sprayer. The nozzles can be moved as a group either in a horizontal or vertical direction. Thus some control is obtained over the spray as well as providing a method to get the tops with the higher nozzles. In comparison with a speed sprayer and a high pressure machine using two brooms, this vertical boom definitely gave more uniform coverage with more lead arsenate in the tops.

In conclusion it may be stated that the speed sprayer offers a means of covering more trees with less labor. However, it does seem that some improvements are necessary in order to get satisfactory coverage in the tops particularly in areas where codling moth is such an acute problem. Some

Mono-
32.
of Oils,
921.
32).

Note (a) All trees were sprayed with dormant Tar-Lub. oil spray, using the Speed-sprayer. No time or gal. record for dormant spray. We were learning to use the sprayer.

(b) In sprays 1-2-3 the Speed sprayer used about 2 to 3 gal. more of spray per tree than did the broom spraying. Broom spraying had 1 man on tank using an 8 nozzle broom and 1 man as tractor driver. In 5-6-7 and 8th sprays man was used with the broom outfit. He was using a single gun from the ground, and in these sprays the gals. per tree are increased. In spray 5, gal. per tree were about equal for each method.

In sprays 5-6-7 and 8, the Speed sprayer gals. per tree increased 3 to 4 gals.

(c) The time of spraying between the 2 methods is in favor of the Speed sprayer, with the Speed-sprayer between 2.5 and 3 times as fast. When the 3rd man was added to the broom spraying method, the cost per tree of labor steps up 1/3 more.

The Speed sprayer 2 man crew was—1 man on tank supply truck and one man on tractor operating tractor and Speed-sprayer.

(d) During the 2-3-4 and 5th spray period, 5/5/43 to 6/14/43 many rainy days marked the period. There were 16 rainy days between 5/5/43 and 6/14/43. No. of gals, for different applications vary in each method because of wind conditions. The variation seems to be uniform to a large extent with both methods. With the amount of time it rained between 5/5/43 and 6/14/43 the speed up in spraying of the Speed-sprayer made it possible to cover the entire 100 acres in fruit in a manner that resulted in a right clean crop at harvest time.

(e) Several light frosts in early April. Mild, weak Dry-Lime sulfur spray used in spray 3-4-5 to reduce russet damage as much as possible.

Sept. 10, 1943—Speed-sprayer—559 lugs dumped. Packed 513 bushel 46 lugs loss to pack. McIntosh. Just a packing house run not a given row.

Ciders (under 2¼)	54 bu.	10.5%
Packing house culls 2½ up)	98 bu.	19.1%
Commercial pack (2¼-3)	361 bu.	70.3%

Sept. 14, 1943—Speed-sprayer count row—254 lugs. Packed 215 bu. 39 lugs loss to pack. McIntosh.

Ciders	19 bu.	8.8%
Packing house culls	59 bu.	27.4%
Commercial pack	137. bu	63.7%

Sept. 14, 1943—Speed-sprayer—Just a run. No count of lugs dumped. 314 packed bu. McIntosh.

Ciders	42 bu.	13.3%
Packing house culls	81 bu.	25.7%
Commercial pack	191 bu.	60.8%

Sept. 16, 1943 & 20, 1943—Broom hand spraying count row—539 lugs. Packed 320. 39 lugs loss to McIntosh.

Ciders	18 bu.	5.6%
Packing house culls	64 bu.	20.0%
Commercial pack	238 bu.	74.3%

The speed sprayer unit averaged in the first four sprays 2.36 times as fast as was the spraying done by the two men operating the 35-gallon high pressure pump with a 400-gallon tank. In the four cover sprays, the speed sprayer unit averaged 2.48 times as fast with a two-man crew, as did the high pressure sprayer unit with a three-man crew.

Samples for lead analysis were taken immediately after each cover spray from both tops and bottoms of each of six trees in each plot. These data were averaged and expressed as milligrams of lead per square meter with both surfaces considered on the leaves. The results of these studies which are summarized in table I show that the high pressure equipment gave approximately equal deposits in top and bottom indicating a fairly uniform coverage. However, the speed sprayer was somewhat disappointing in this respect. This machine put more material on the bottoms and considerably less on the tops. Scab control was better in the tops with the high pressure sprayer. There was no codling moth infestation in this orchard, so a comparison of control of this insect was not possible.

The lead deposit in different parts of the other branches of the upper third of the tree did not seem to vary significantly on different sides with either type of equipment. Packing house grading records on the McIntosh gave an average of 9.6 percent scab with the speed sprayer against 4.8 percent on the high pressure sprayed rows. The speed sprayer applied slightly more material as indicated by an average of 17.6 gallons per tree against 15.3 for the high pressure.

One approach to this problem of better coverage with less labor has been developed at the Delaware station (2). Standard type gun nozzles are mounted on a mast attached to the back of a regular high pressure sprayer. The nozzles can be moved as a group either in a horizontal or vertical direction. Thus some control is obtained over the spray as well as providing a method to get the tops with the higher nozzles. In comparison with a speed sprayer and a high pressure machine using two brooms, this vertical boom definitely gave more uniform coverage with more lead arsenate in the tops.

In conclusion it may be stated that the speed sprayer offers a means of covering more trees with less labor. However, it does seem that some improvements are necessary in order to get satisfactory coverage in the tops particularly in areas where codling moth is such an acute problem. Some adaptions are also being developed for the regular high pressure equipment which will eliminate some of the labor now required and possibly cost considerably less.

(1) Kadow, K. J. and S. L. Hopperstead—An evaluation of new spray equipment. Trans. Peninsula Hort. Soc. 32: 13-18,1942.

(2) Hopperstead, S. L., M. W. Goodwin, and Paul L. Rice (abstr.) Phytopath 33:1114, 1943.

(3) Weber. A. L. and H. C. McLean—Spray coverage of apple trees as affected by different methods of application. Am. Soc. Hort. Sci. 42:285-288. 1943.

TABLE I

Comparison between Speed Sprayer and High Pressure Sprayer
Pennsylvania State College—1943

Milligrams of lead per square meter of surface

Date	Stayman Winesap				McIntosh			
	Top of tree		Bot of tree		Top of tree		Bot. of tree	
	Speed Spr.	High Pres.	Speed Spr.	High Pres.	Speed Spr.	High Pres.	Speed Spr.	High Pres.
Leaves—6/1	2.5 **	37.3	63.1 **	31.2	11.7 **	24.7	41.0 **	27.8
" 6/14	20.5 **	60.4	81.0	70.5	13.6 **	55.1	66.4 **	49.6
" 6/28	37.5 **	74.8	87.6	85.6	18.9 *	37.8	87.6 **	68.3
" 7/23	34.6 **	69.6	71.3	68.7	18.6 **	57.2	87.2 **	65.2
" Mean	23.8	60.5	75.8	64.0	15.7	43.7	70.6	52.7
[illegible] -6/28	7.5	13.2	9.1	15.9	2.7 **	6.7	9.1	7.6
% scab-leaves—7/13					6.9**	2.3	0.3	0.3

*—Significant difference between two figures, odds 19:1.
**—Significant difference between two figures, odds 99:1.

1943 Test Spraying McIntosh and Stayman—Broom-spraying vs. Speed sprayer spraying.

	35 gal. Bean pump broom		Speed sprayer	
1st spray-delayed dormant—4/26/43 L-Sl. 5 gal.-100	2 men 140 min.	900 gal. 69 trees 13 per tree Time per tree 2.02 min.	2 men 165 min.	300 gal. 199 trees 15+ per tree Time per tree .82 min.
2nd spray-Pre-pink to Pink—5/5/43 L.S.1.5 gal.-3 lb. lead	2 men 130 min.	850 gal. - 69 trees 12+ per tree Time per tree 1.9-.	2 men 16 min.	300 gal. 216 trees 14- per tree Time per tree .76+.
3rd spray-Full bloom—5/12/43 Dry lime S. 5 lb-100	2 men 120 min.	800 gal. - 69 trees 11+ per tree Time per tree 1.7+.	2 men 255 min.	4100 gal. 297 trees 13.8 gal. per tree Time per tree .75+.
4th spray-Petal Fall—5/19/43 Dry L.S. 4 lb. + 3 lb. hydrate lime 3 lb. lead 1 part nicotine-800	2 men 120 min.	950 gal. - 69 trees 13.8 per tree Time per tree 1.73+.	2 men 150 min	3000 gal. 191 trees 15.7 gal. per tree Time per tree .78.
5th spray. First cover—6/1/43 Dry L-S 4 lbs. 3 lb. hydrated lime 3 lb. lead 1 part nicotine - 2000	3 men 180 min. (1 man spraying from ground)	1175 gal. - 69 trees 17 per tree Time per tree 2.6	2 men 145 min.	3350 gal. 191 trees 17 gal. per tree Time per tree .75+.
6th spray. Second cover—6/14/43 Bordeaux 1-3-100 3 lb. lead - 100	3 men 150 min.	1300 gal. - 69 trees 18.8 gal, per tree Time per tree 2.17	2 men 190 min.	3400 gal. 161 trees 21+ gal. per tree Time per tree 1.1.
7th spray. Third cover—5/28/43 Bordeaux 1-3-00 3 lb. lead - 100	3 men 170 min.	1200 gal. - 69 trees 17.4 gal. per tree Time per tree 2.45	2 men 155 min.	3350 gal. 161 trees 20.8 gal. per tree Time per tree .96 min.
8th spray. Fourth cover—7/24/43 Bordeaux 1-3-100 3 lb. lead - 100	3 men 180 min.	1300 gal. - 69 trees 18.7 gal. per tree Time per tree 2.6	2 men 165 min.	3700 gal. 161 trees 23 gal. per tree Time per tree 1.02 min.

. S. Mono-
, 1932.
ysis of Oils,
., 1921.
(1932).

Note (a) All trees were sprayed with dormant Tar-Lub. oil spray, using the Speed-sprayer. No time or gal. record for dormant spray. We were learning to use the sprayer.

(b) In sprays 1-2-3 the Speed sprayer used about 2 to 3 gal. more of spray per tree than did the broom spraying. Broom spraying had 1 man on tank using an 8 nozzle broom and 1 man as tractor driver. In 5-6-7 and 8th sprays man was used with the broom outfit. He was using a single gun from the ground, and in these sprays the gals. per tree are increased. In spray 5, gal. per tree were about equal for each method.

In sprays 5-6-7 and 8, the Speed sprayer gals. per tree increased 3 to 4 gals.

(c) The time of spraying between the 2 methods is in favor of the Speed sprayer, with the Speed-sprayer between 2.5 and 3 times as fast. When the 3rd man was added to the broom spraying method, the cost per tree of labor steps up 1/3 more.

The Speed sprayer 2 man crew was—1 man on tank supply truck and one man on tractor operating tractor and Speed-sprayer.

(d) During the 2-3-4 and 5th spray period, 5/5/43 to 6/14/43 many rainy days marked the period. There were 16 rainy days between 5/5/43 and 6/14/43. No. of gals, for different applications vary in each method because of wind conditions. The variation seems to be uniform to a large extent with both methods. With the amount of time it rained between 5/5/43 and 6/14/43 the speed up in spraying of the Speed-sprayer made it possible to cover the entire 100 acres in fruit in a manner that resulted in a right clean crop at harvest time.

(e) Several light frosts in early April. Mild, weak Dry-Lime sulfur spray used in spray 3-4-5 to reduce russet damage as much as possible.

Sept. 10, 1943—Speed-sprayer—559 lugs dumped. Packed 513 bushel 46 lugs loss to pack. McIntosh. Just a packing house run not a given row.

Ciders (under 2¼)	54 bu.	10.5%
Packing house culls 2½ up)	98 bu.	19.1%
Commercial pack (2¼-3)	361 bu.	70.3%

Sept. 14, 1943—Speed-sprayer count row—254 lugs. Packed 215 bu. 39 lugs loss to pack. McIntosh.

Ciders	19 bu.	8.8%
Packing house culls	59 bu.	27.4%
Commercial pack	137. bu	63.7%

Sept. 14, 1943—Speed-sprayer—Just a run. No count of lugs dumped. 314 packed bu. McIntosh.

Ciders	42 bu.	13.3%
Packing house culls	81 bu.	25.7%
Commercial pack	191 bu.	60.8%

Sept. 16, 1943 & 20, 1943—Broom hand spraying count row—539 lugs. Packed 320. 39 lugs loss to McIntosh.

Ciders	18 bu.	5.6%
Packing house culls	64 bu.	20.0%
Commercial pack	238 bu.	74.3%

Oct. , 1943—Speed-sprayer Count Row—340 lugs. Packed 301 bu. 39 lugs loss to pack. Stayman.

Ciders	4 bu.	1.3%
Packing house culls	53 bu.	7.6%
Commercial pack	244 bu.	81.7%

Oct. , 1943—Broom hand spraying Count row. 186 lugs. Packed 172. 14 lugs loss to pack. Stayman.

Ciders	3 bu.	1.7%
Packing house culls	20 bu.	11.6%
Commercial pack	149 bu.	86.6%

NOTE: It is necessary to make a statement regarding the packing house culls. Due to the fact that any U. S. grade of apples was ruling at a high price during the fall of 1943, packing house labor was instructed to sort out every speck or spot and place it in the packing house cull pack. We felt that if prices for U. S. grades continued at a high figure throughout the season we wanted to be sure that our commercial pack was a pack of good clean apples. Much of our pack was not sorted for color and was marked U. S. Commercial. About half of the sorters were green help so we had to insist that any blemished apple was a packing house cull as far as we were concerned.

Mr. Richard Williams was working for us in the packing house at the time of packing. Mr. Williams has had experience in Federal inspection, having worked for Don James of the State Bureau of Markets. Knowing that our packing house culls were right good apples, we had Mr. Williams resort 2 bushel of packing house culls to determine the reason for the apples having been placed in the packing house cull baskets. Packing house culls were "run" from the bin and not picked off the belts. This "run" caused, we know considerable bruising, and accounts for the high percentage of mechanical injury Mr. Williams found in the packing house cull packages.

Broom Spraying			**Speed Spraying**		
U. S. Commercial	2lb	2.4%	U. S. Commercial	2.0lb	2.2%
U. S. Utility	12lb	14.3%	U. S. Utility	7.5lb	8.3%
Mechanical injur.	50lb	59.5%	Mechanical injur.	49.0lb	54.4%
Scab	20lb	23.8%	Scab	31.5lb	35.0%
2 bu. fruit wt.	84lb		2 bu. fruit wt.	90.0lb	

In this grading any blemishes and color requirements were considered in making the Utility number of pounds. No worms were found in William's sorting. It is possible and probable that limb-rubs, insect damage, cedar-rust damage, as well as some scab damage, found its way into the utility percentage in that it would be too great for permitting the fruit to be in the commercial or better grades. Under scab in William's sorting undoubtedly enough was present to keep the individual specimen out of a utility pack. Mechanical injuries includes russet damage.

No breakdown was made of Stayman packing house culls. Dr. Miller, of the Botany Department, had made field counts that showed very little scab injury.

This tabulation was made by F. N. Fagan, Department of Horticulture, The Pennsylvania State College, State College, Penna.

C. S. Mono-
Co., 1932.
nalysis of Oils,
Co., 1921.
-7 (1932).

Cabbage Maggot Tests in 1943[1]

L. E. Dills, D. E. H. Frear and H. L. King[2]

In the spring of 1943, the scarcity and high price of mercury emphasized the desirability of testing a number of mercury substitutes for control of the cabbage maggot. Field experiments were conducted at the Pennsylvania State College to test some possible substitutes.

Since facilities were limited and it was necessary to test a large number of materials in a small space with a considerable degree of accuracy, cabbage plants could not be used. Radishes* offered the most practical solution of the problem, since they required little space and were a host plant preferred by this maggot. The most apparent disadvantage was that they are injured by some materials which may safely be used on cabbage.

Thirty-five different treatments were used in two field tests. These included 22 different materials, some at several concentrations. In the first experiment most of the treatments were replicated six times. In the second experiment all treatments were replicated seven times. The plots were randomized to eliminate any differences in location. Counts were made in 250 plots and approximately 9000 radishes were cut and examined for maggots.

Only one application was made and with the exception of corrosive sublimate and dichloroethyl ether all the materials were used as dusts. All of the materials tests as dusts (except calomel) were impregnated on bentonite. Calomel was used as mechanical mixtures with lime and with bentonite. A 2-quart plunger type duster was used in making the application. Dust was used in the tests because truck growers have expressed a preference for dusts, and they have requested specifically a material that can be applied to cabbage stems before setting. Even effective materials that require two or three applications, especially of a liquid, may be of questionable practical use under some conditions. It was with this viewpoint that these tests were conducted.

In Experiment 1 white icicle radishes were used and the treatments were made when eggs were being laid and the plants had one pair of primary leaves. This is the usual time for making such applications.

---

[1] Authorized for publication on February 28, 1944, as paper No. 1223 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Agricultural Experiment Station, The Pennsylvania State College.

* The radish and cabbage plants used in these experiments were supplied by Dr. W. B. Mack and Dr. C. E. Myers of the Department of Horticulture.

Experiment 1

Treatment	Plant Injury	Per Cent Not Infested
Calomel 10%	None	84.7
Paradichlorobenzene 10%	Very slight	62.4
Paradichlorobenzene 20%	Very slight	45.1
Paradichlorobenzene 40%	Slight	48.8
Dichloroethyl ether 1-400	None	74.8
Dichloroethyl ether 1-800	None	65.5
Naphthalene 10%	Very slight	54.6
Naphthalene 20%	Slight	58.0
Naphthalene 40%	Slight	55.3
Check (no treatment)	None	50.1
2-ethyl-pyridine sulphate 10%	Very severe	94.6
2-n-hexyl pyridine sulphate 10%	Moderate	74.4
2-mixed undecyl pyridine sulphate 10%	Moderate	73.4
4-n-propyl pyridine sulphate 10%	Severe	65.4
4-n-amyl pyridine sulphate 10%	Moderate	58.8
Quinoline sulphate 10%	Slight	67.4
Hexachlorethane 10%	Very slight	58.2
Hexachlorethane 20%	Slight	47.0
Hexachlorethane 40%	Slight	49.8
Phenothiazine 10%	Moderate	56.0
Phenothiazine 20%	Severe	58.8
(1) Mathieson alkali weed killer 10%	Slight	62.8
(1) Koppers dormant tar oil 10%	Very severe	100
(1) Beta Naphthol 10%	Dead	100
(1) Penta capryl laurylamine tripolyphosphate 10%	Slight	60.3
(1) Kerosene phosphine sulphide 10%	None	48.3
(1) Chlorophenyl thiophosphonic acid 10%	Slight	47.3
(2) Cresylic acid 10%	Dead	100
(1) Potassium sulphide 10%	Very slight	68.1

Least significant difference between treatments (6 plots) 16 per cent
(1) = 2 plots (2) = 4 plots

In Experiment 2 scarlet globe radishes were used and the application was made almost a week later than Experiment 1. This was done because growers frequently fail to apply the material at the proper time and inquire as to the effectiveness of later applications.

Experiment 2

Treatment	Plant Injury	Per Cent Not Infested
Calomel 4% with bentonite	None	56.9
Calomel 10% with bentonite	None	65.9
Calomel 4% with lime	None	44.6
Calomel 10% with lime	None	50.6
Dichloroether ether 1-200	None	89.4
Dichloroether ether 1-400	None	80.5
Corrosive sublimate 1-1000	Slight	74.1
Check	None	41.7
Phenothiazine 40%	Moderate	46.0
Naphthalene 40%	Very slight	67.0
Paradichlorobenzene 40%	Very slight	48.5
Hexachlorethane 40%	Very slight	48.5
Koppers dormant tar oil 5%	Moderate	70.8
Calcium sulphide 10%	None	56.6

Least significant difference between treatments 10 per cent.

When applied early a 10 per cent calomel dust was the best safe treatment used for radishes. When applied late it was much less effective. Bentonite and calomel gave much better results than lime and calomel.

When applied late the best safe material used on radishes was dichloroethyl ether. The results suggest that corrosive sublimate solution is a better late treatment than calomel dust when applied one week later than the optimum date.

Paradichlorobenzene, hexachlorethane, phenothiazine, 4-n-amyl pyridine sulphate, kerosene phosphine sulphide, Mathieson alkali weed killer, pentacapryl laurylamine tripolyphosphate, and chlorophenyl thiophosphonic acid at the concentrations used showed little or no toxicity to the cabbage maggot. Although not significantly toxic, potassium sulphide, and 4-n-propyl pyridine sulphate appeared to have some effect at the concentrations used in these tests. Naphthalene at 40 per cent in experiment two, 2-n-hexyl pyridine sulphate, 2-mixed-undecyl pyridine sulphate, quinoline sulphate, and calcium sulphide showed considerable toxicity to this insect but did not give satisfactory results. Koppers dormant tar oil and 2-ethyl pyridine sulphate were found to prevent insect injury but caused serious damage to the radish plants. Beta naphthol and cresylic acid dusts at 10 per cent killed the plants so no reliable information could be obtained.

Several of the new materials that had been found to be most effective in preventing maggot damage to radishes were given preliminary tests on cabbage. Koppers dormant tar oil, 2-ethyl pyridine sulphate and 2-n-hexyl pyridine sulphate dusts at 10 per cent were applied to the dampened stems of late cabbage before setting. Records kept for more than two months indicated that there were no harmful effects.

These trials on both radishes and cabbage indicate that certain of the materials tested show promise of being used as substitutes for the mercury compounds. Further work will be necessary to establish the exact conditions for their most efficient use. Plans are being made to continue this work in 1944.

Reprinted from Pennsylvania Vegetable Growers' News
State College, Pa.
Volume IX No. 1 March 1944

Reprinted from THE JOURNAL OF NUTRITION
Vol. 27, No. 5, May, 1944

EFFICACY OF VITAMIN D FROM DIFFERENT SOURCES FOR TURKEYS [1]

ROBERT V. BOUCHER

Department of Agricultural and Biological Chemistry, The Pennsylvania State College
State College

TWO FIGURES

(Received for publication December 20, 1943)

It is well known that turkeys have a higher requirement for certain specific nutrients than chickens and this is particularly true of vitamin D as first pointed out by Scott, Hughes and Loy ('32). Since then, however, there has been considerable disagreement among those who have attempted to measure the amount of vitamin D required by young growing turkeys. Baird and Greene ('35) using a single vitamin D supplement, a fortified cod liver oil, determined the levels required to prevent rachitic symptoms in growing chicks, poults and pheasants. They reported that turkeys require a minimum of 60 to 70 U.S.P. units of vitamin D per 100 gm. of feed during the first 12 weeks of life. A careful study of this work indicated that these authors may not have obtained the maximum response in calcification and growth from even the highest level of vitamin D fed in their turkey experiments and that, therefore, the requirement for maximum response could have been distinctly higher. Furthermore, the potency of their supplement was originally established in Steenbock rat units and the question of the conversion factor used in calculating U.S.P. units would also tend to raise doubt as to the true value of the units fed by them as compared with A.O.A.C. chick units used by later workers.

Jukes and Sanford ('39) were the first to attempt the quantitative determination of the response of young turkeys on a rickets-producing diet to a standard vitamin D substance, namely, U.S.P. reference cod liver oil no. 1, the potency of which was definitely known in terms of A.O.A.C. chick units. They also fed in the same experiments a sardine oil, fortified with fish liver oils, which was assayed with chicks against the reference cod liver oil. They found that levels of the reference cod liver oil up to and including 120 A.O.A.C. units per 100 gm. of diet produced unsatisfactory growth and calcification to 4 weeks of age. Unfortunately, they did not feed higher levels but extrapolation of their curve of response indicated that more than 200 units would be needed for maximum calcification. Surprisingly, they noted that levels of the fortified sardine oil, equivalent to the reference oil in A.O.A.C. chick units, produced a greater calcifying effect in the turkeys. This led them to suggest the possibility of a difference between chicks and turkeys in their response to different sources of vitamin D and they properly pointed out the difficulty of expressing the requirements of turkeys in terms of chick units.

---

[1] Authorized for publication on December 13, 1943, as paper no. 1213 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The author is indebted to Prof. H. C. Knandel of the Poultry Husbandry Department and to Dr. J. Waddell of E. I. du Pont de Nemours and Company for assistance and advice.

Hammond ('41) in a brief paper reported the feeding of reference cod liver oil, a fortified cod liver oil and activated animal provitamin D to poults during the first 4 weeks of life as supplements to a rachitogenic diet. In sharp contrast with the findings of Jukes and Sanford ('39) he found that 80 A.O.A.C. chick units from the reference cod liver oil produced a maximum effect on body weight and bone calcification. His data on the other two supplements are of less value in estimating the efficacy of each since he relied on the manufacturer's claims for the A.O.A.C. chick unit potency. In addition, he presented no information on the source of the vitamin D used to fortify the cod liver oil, and the levels fed of activated animal provitamin D, except one, were unreasonably high. It is interesting to note, however, that the 50-unit level of activated animal provitamin D gave a response approximately equal to 80 units of the other two supplements.

After the work here reported had been started, the publication of Carver and Rhian ('42) appeared. These workers fed a fortified cod liver oil at 8 levels (50 to 200 units) and activated animal provitamin D at 1 level (100 units) to duplicate groups of poults receiving a rachitogenic diet. Both supplements were assayed by the authors and the dosage was expressed in A.O.A.C. chick units. No reference cod liver oil was fed. Carver and Rhian concluded that 80 A.O.A.C. chick units per 100 gm. of feed from either of the supplements produced satisfactory growth and calcification in young turkeys. No information was given as to the source or composition of the fortified oil.

The primary object of the experiments here described was to compare the efficacy of vitamin D from different sources for turkeys, particularly when fed on the basis of equal potency, measured in A.O.A.C. chick units. It appeared also that the observations of Jukes and Sanford ('39) were worthy of further investigation and that additional work of this nature might help resolve the lack of agreement as to the requirement of young turkeys for this essential nutrient.

EXPERIMENTAL

The percentage composition and analysis of the basal diet are given in table 1. This diet was formulated with the idea of supplying optimum amounts of all the essential nutrients with the exception of vitamin D. It resembled typical turkey starting mashes except that casein was used in place of meat scraps or fish meal in order to avoid possible contamination with vitamin D from animal sources. The dehydrated alfalfa meal was of high quality as shown by analyses indicating a carotene content equivalent to 92,400 U.S.P. units of vitamin A per pound and a riboflavin content of 5,630 µg. per pound. A vitamin D assay of the alfalfa meal using rats as experimental animals showed that it contained between 363 and 454 U.S.P. units per pound. This amount was considered negligible since it was presumed to be the ineffective activated plant sterol type. The results proved this to be true.

TABLE 1

Percentage composition and analysis of basal diet.

Ground yellow corn	25.9	Ground oats	10.0	Dried brewers' yeast	6.0
Soybean oil meal	12.0	Dry skimmilk	8.0	Ground limestone	2.0
Wheat bran	10.0	Dehydrated alfalfa meal	7.5	Corn oil Mazola)	2.0
Wheat middlings	10.0			Sodium chloride	0.5
		Commercial casein	6.0	Manganese sulfate	0.1

Protein (N x 6.25) %	24.56	Crude fiber %	5.18
Moisture %	9.74	Calcium %	1.05
Ash %	5.46	Phosphorus %	0.63
Ether extract %	5.43	Manganese %	0.01

Riboflavin — micrograms per 100 gm.	575
Vitamin A — International units per 100 gm.	1724

A preliminary trial employing 3 groups of poults was conducted for the purpose of testing the suitability of the basal diet described in table 1 and of securing some indication of the response which might be obtained to two supplements representing diverse sources of vitamin D. One group of poults received the basal diet alone, the second received as a supplement to the basal 115 A.O.A.C. units per 100 gm. of diet from fortified sardine oil, while the third received as a supplement 105 A.O.A.C. units per 100 gm. of diet from irradiated 7-dehydrocholesterol. The groups were continued for 4 weeks on these diets. On the twenty-eighth day the survivors were killed and bone ash determined by the method described below. Of the ten poults started on the basal, only three survived; these had an average bone ash of 30.3%. Nine poults that received the sardine oil had an average bone ash of 45.1% while the eight poults that received the irradiated 7-dehydrocholesterol had an average bone ash of 49.0%. There was no mortality in the two groups of poults to which supplements were given.

These preliminary results indicated that the basal diet was satisfactory for producing rickets and that there might well exist distinct differences between different vitamins D in their ability to prevent rachitic manifestations in poults. Thus encouraged, a more extensive and quantitative comparison of four vitamin D supplements from different origins was undertaken. Two separate experiments were carried out in which these supplements were fed at various levels, experiment 2 being planned and carried out after the results of experiment 1 had been carefully studied.

The vitamin D supplements employed were: (1) U.S.P. reference cod liver oil no. 2; (2) a corn oil solution of an irradiated animal sterol; (3) a corn oil solution of irradiated 7-dehydrocholesterol; and (4) a fortified sardine oil.[2] These materials were chosen for the reason that they were representative of vitamins D of diverse origin and also of those largely used in modern poultry feeds. The reference cod liver oil was a standard test substance of known vitamin D content and in addition was presumed to be representative of cod liver oils in general. The fortified sardine oil was supplied with the assurance that all of its vitamin D activity came from fish oil sources. It was assumed that its vitamin D activity would be representative of sardine oil plus whatever high potency liver oil was used for increasing its D content. The irradiated animal sterol was prepared by irradiating with ultraviolet light the purified sterol mixture isolated from a species of mussel. The exact chemical nature of the vitamin D which it contained has not been reported. The irradiated 7-dehydrocholesterol, on the other hand, contained a single known vitamin D, namely D_3. Both irradiated products have come into wide commercial use under the designation of D-Activated Animal Sterol.

The various levels of the supplements were introduced into the diet by pre-mixing with the corn oil component. In order to establish their potency with accuracy the irradiated animal sterol, the irradiated 7-

dehydrocholesterol, and the fortified sardine oil were carefully assayed by the A.O.A.C. chick method in three separate laboratories. The results of the separate assays and the average value assigned to each supplement are summarized in table 2. It is believed that the assigned values have a high degree of accuracy.

White Holland and Bronze turkey poults were used in these investigations, representatives of both breeds being included in each group. Twelve to fourteen poults in each group were started when 1 day old and kept for 28 days in electrically heated battery brooders equipped with hardware cloth floors and automatic waterers. The brooders were located in a laboratory from which all direct sunlight was excluded by covering the closed windows with heavy brown paper. The experimental diets and tap water were supplied ad libitum.

TABLE 2

A.O.A.C. chick assays of the vitamin D concentrates.

LABORATORY [1]	IRRADIATED ANIMAL STEROL	IRRADIATED 7-DEHYDROCHOLESTEROL	FORTIFIED SARDINE OIL
	Units per gm.	*Units per gm.*	*Units per gm.*
1	445 (4)[2]	340 (4)	435 (4)
2	404 (4) 403 (3)	342 (4)	408 (4)
3	423 (3) 399 (3)	415 (3) 411 (3)	450 (3)
Assigned values	415	375	430

[1] Laboratory 1, the Department of Agricultural and Biological Chemistry of The Pennsylvania State College. Laboratory 2, the Laboratory of Vitamin Technology, Chicago. Laboratory 3, the Biological Laboratory of E. I. du Pont de Nemours and Company, New Brunswick, N. J.

[2] The figure in parentheses following each assay value represents the number of groups of chicks used in that assay.

At the conclusion of each experiment the surviving poults were killed and the tibias removed for ash determination. This was done essentially as outlined in Official and Tentative Methods of Analysis ('40), except that the extraction was continued for 30 hours with 95% ethyl alcohol followed by 30 hours with ethyl ether. The extracted bones were dried to constant weight at 103°C. and then ashed by placing the crucibles in a cold furnace and raising the temperature slowly to 400°C. The total time required for this method of ashing approximated 16 hours, during the last three of which the temperature was increased to 800°C. While the bones were individually ashed only the average value for each group has been recorded in the subsequent tables.

---

[2] Thanks are expressed to E. Fullerton Cook, Chairman of the Committee of Revision of the Pharmacopoeia of the United States of America for the reference cod liver oil, to J. Waddell of E. I. du Pont de Nemours and Company for both the irradiated animal sterol and irradiated 7-dehydrocholesterol, and to T. D. Sanford of F. E. Booth Company for the fortified sardine oil.

RESULTS AND DISCUSSION

In the first experiment nineteen groups of poults were used. These were divided as follows: poults in one group received the basal diet alone; six groups of poults received the basal plus levels of reference cod liver oil ranging from 20 to 200 units per 100 gm. of diet; and four groups of poults were placed on each of the other three supplements. The levels at which these supplements were fed were chosen in the hope that they would yield responses which could be compared quantitatively with those given by the reference oil. The range originally planned was from 20 to 120 A.O.A.C. units of each supplement per 100 gm. of diet but the final assigned A.O.A.C. potency, calculated after the turkey experiments had been started, modified these values slightly. Detailed information on the results obtained from experiment 1, such as mortality, body weight, dry weight of bone, and bone ash percentage is summarized in table 3.

TABLE 3

Summary of experiment 1.

GROUP	SUPPLEMENT PER 100 GM. BASAL DIET			MORTALITY DUE TO RICKETS	RESULTS AT 4 WEEKS OF AGE			
	Source	mg.	A.O.A.C. units	%	No. of poults	Body weight gm.	Tibia weight gm.	Tibia ash %
1	None	0	0	92	1	112	.4221	25.5
2	Reference	174	20	92	1	192	.5780	32.4
3	cod liver	348	40	67	4	209	.6776	32.8
4	oil	696	80	0	12	260	.8352	35.0
5		1043	120	0	12	307	.9915	42.7
6		1391	160	0	12	285	.9199	42.2
7		1739	200	0	13	302	.9780	44.6
8	Irradiated	47	19	42	7	176	.5885	31.5
9	animal	93	39	9	10	247	.7770	37.4
10	sterol	186	77	0	13	265	.8069	47.7
11		279	116	0	12	276	.8703	48.7
12	Irradiated	47	17	64	4	186	.5845	31.0
13	7-dehydro-	93	35	8	11	217	.7302	34.8
14	cholesterol	186	70	0	13	335	1.0677	45.8
15		279	105	0	13	299	.9304	46.1
16	Fortified	44	19	69	4	187	.5648	27.4
17	sardine	89	38	42	7	210	.6933	31.3
18	oil	178	76	22	7	242	.7442	38.3
19		267	115	0	12	294	.9261	41.2

In order to confirm the surprising differences in efficacy noted among the supplements in the first experiment, a second was carried out. In this second experiment 20 groups of poults were used, these being distributed as in the first except that 7 groups of poults received reference cod liver oil. The levels fed of each supplement were adjusted somewhat from those used in the first experiment in order to facilitate quantitative comparisons. In addition, higher levels of reference cod liver oil were fed because of the evidence that 200 units did not produce maximum bone ash in the first experiment. Details and results of experiment 2 are presented in table 4.

TABLE 4

Summary of experiment 2.

GROUP	SUPPLEMENT PER 100 GM. BASAL DIET			MORTALITY DUE TO RICKETS	RESULTS AT 4 WEEKS OF AGE			
	Source	mg.	A.O.A.C. units	%	No. of poults	Body weight gm.	Tibia weight gm.	Tibia ash %
1	None	0	0	88	1	137	.7106	28.3
2	Reference	348	40	10	9	226	.6112	33.5
3	cod liver	522	60	0	8	261	.7121	38.7
4	oil	696	80	0	8	295	.8676	40.5
5		870	100	0	10	284	.8411	42.6
6		1217	140	0	9	279	.8208	46.4
7		1739	200	0	10	302	.9825	47.1
8		2609	300	0	8	302	.9278	47.3
9	Irradiated	93	39	0	9	231	.6465	40.8
10	animal	140	58	0	8	264	.7394	46.2
11	sterol	186	77	0	9	237	.6829	49.1
12		233	97	0	5	287	.8478	49.1
13	Irradiated	93	35	0	7	203	.5869	39.1
14	7-dehydro-	140	52	0	8	234	.6563	42.5
15	cholesterol	186	70	0	7	282	.8747	47.6
16		233	87	0	7	252	.7505	49.1
17	Fortified	133	57	0	8	230	.6210	41.2
18	sardine	222	96	0	9	228	.6564	44.7
19	oil	311	134	0	8	283	.8650	47.5
20		444	191	0	9	326	.9388	49.8

The data representing the bone ash response obtained in experiments 1 and 2 are presented graphically in figures 1 and 2, respectively, the percentage bone ash being plotted against the number of A.O.A.C. units fed of each supplement.

From a study of the results of these experiments it was quite clear that the vitamins D in the two irradiated products were much more effective in promoting calcification in turkey poults than those of the fish oils when compared on the basis of the A.O.A.C. chick units fed. The results of the two experiments agreed very well.

An inspection of the graphs indicates that the response curves of the two irradiated products have a different slope from the response curves of the fish oils. It is obvious, therefore, that in attempting to give numerical expression to the differences in efficacy under these conditions a different value will be obtained for every level of response at which the comparison is made. For the present purpose a straight line of best fit has been drawn (by inspection) through the cod liver oil responses in each experiment. By placing on these approximated lines the equivalent bone ash responses of the other supplements the corresponding units have been read for each level fed. By comparing these "response" units with the number of A.O.A.C. units actually fed the

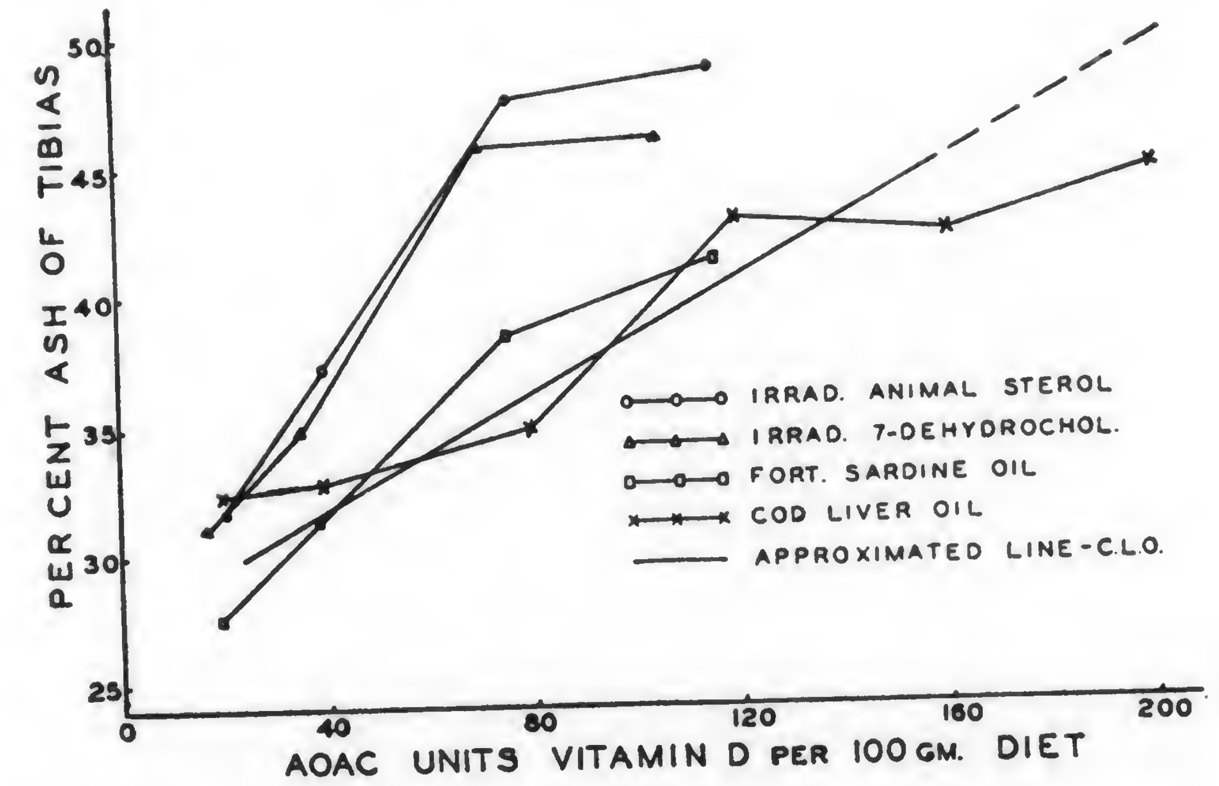


Fig. 1 Bone ash responses of poults fed vitamin D from different sources. Experiment 1.

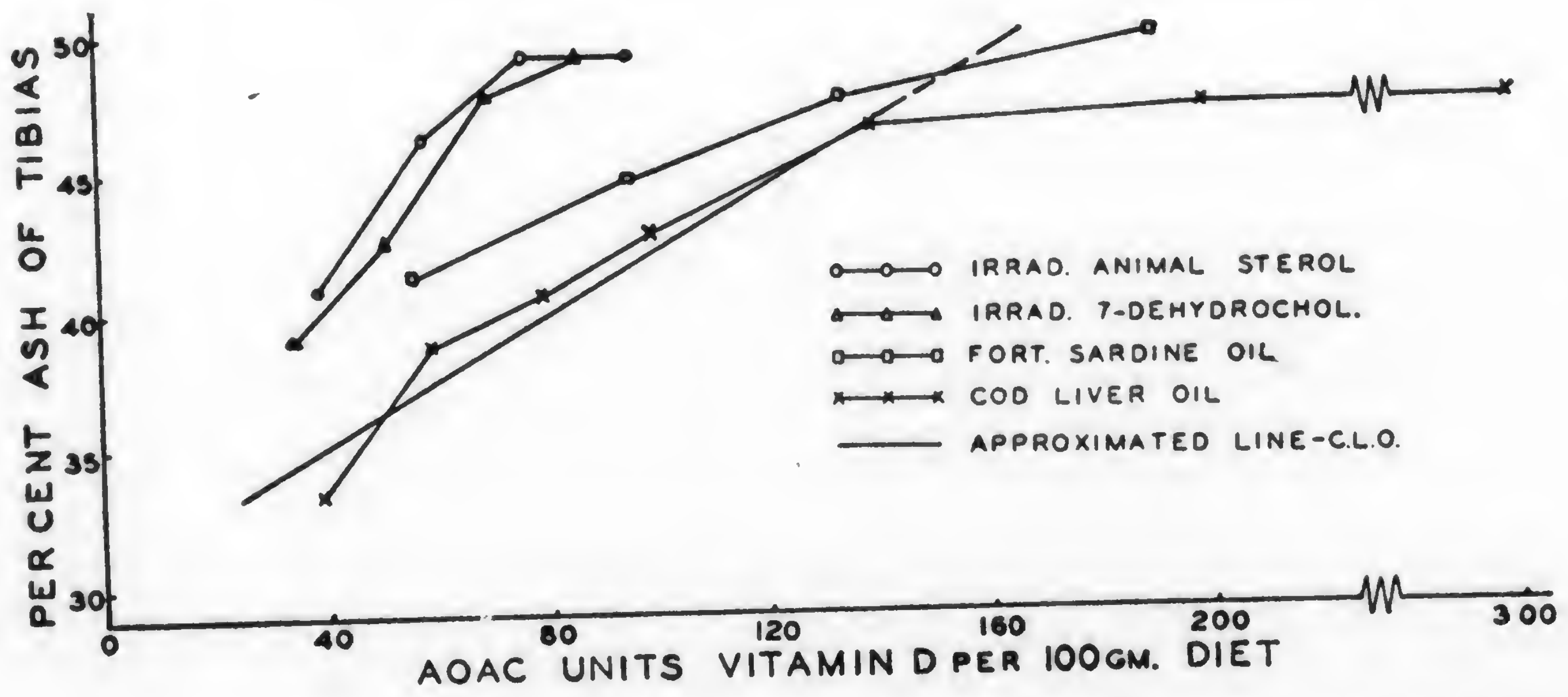


Fig. 2 Bone ash responses of poults fed vitamin D from different sources. Experiment 2.

average efficacy ratio of each supplement as compared with the reference oil was calculated. These calculations for both experiments have been summarized and averaged in table 5.

TABLE 5

Efficacy ratio of the three supplements compared with reference cod liver oil.

SUPPLEMENT	GROUP NO.	TIBIA ASH %	A.O.A.C. UNITS FED	A.O.A.C. UNITS FROM C.L.O. LINE	EFFICACY RATIO CLO = 1
		Experiment 1			
Irradiated animal sterol	8	31.5	19	38	2.0
	9	37.4	39	91	2.3
	10	47.7	77	183	2.4 [1]
	11	48.7	116	192	1.7 [1]
					Average 2.1
Irradiated 7-dyhydro-cholesterol	12	31.0	17	33	1.9
	13	34.8	35	68	1.9
	14	45.8	70	160	2.3 [1]
	15	46.1	105	170	1.6 [1]
					Average 1.9
Fortified sardine oil	16	27.4	19	..	..
	17	31.3	38	35	0.9
	18	38.3	76	99	1.3
	19	41.2	115	125	1.1
					Average 1.1
		Experiment 2			
Irradiated animal sterol	9	40.8	39	87	2.2
	10	46.2	58	134	2.3
	11	49.1	77	158	2.1
	12	49.1	97	158	1.6 [1]
					Average 2.1
Irradiated 7-dehydro-cholesterol	13	39.1	35	73	2.1
	14	42.5	52	106	2.0
	15	47.6	70	145	2.1 [1]
	16	49.1	87	158	1.8 [1]
					Average 2.0
Fortified sardine oil	17	41.2	57	91	1.6
	18	44.7	96	120	1.3
	19	47.5	134	145	1.1 [1]
	20	49.8	191	164	0.9 [1]
					Average 1.2

[1] Value obtained by extending approximated straight line for the cod liver oil.

By this procedure it will be seen that the efficacy ratio for the irradiated animal sterol averaged 2.1 in both experiments, that of the irradiated 7-dehydrocholesterol 1.9 and 2.0, and that of the fortified sardine oil 1.1 and 1.2. Obviously, for the reasons already mentioned, the ratios calculated at the high bone ash responses are relatively less valuable from a quantitative standpoint since it was only by extrapolating the straight line of the cod liver oil beyond the actual values given by this supplement that comparisons could be made at these levels. Actually, the reference oil, even when fed at 300 units per 100 gm. of diet never gave as high bone ash values as the other supplements. Consequently, if one compares the efficacy of the supplements at the level required to give maximum calcification, values appreciably greater than the averages given in table 5 would be indicated.

This suggests that turkeys, at least under the conditions of these experiments, utilize certain forms of vitamin D with degrees of efficacy that differ from chickens and exhibit a higher degree of species specificity than chickens. Certainly, it can be said that the A.O.A.C. chick unitage is not necessarily a true measure of the value of a vitamin D source for turkeys.

The results here reported confirm those of Jukes and Sanford ('39) in regard to the relative efficacy of reference cod liver oil and fortified sardine oil. It is to be remembered, however, that Jukes and Sanford ('39) used U.S.P. reference cod liver oil no. 1. While reference cod liver oil is measurably less effective than fortified sardine oil, on the basis of chick unit content, the slopes of their response curves are similar whereas both are distinctly different from the slopes of the irradiated products curves. If reference cod liver oil and fortified sardine oil can be assumed to be representative of fish oils generally, then 200 units per 100 gm. of diet from such sources may be considered an approximation of the "requirement" of turkey poults. In contrast, approximately 80 units from irradiated animal sterol or irradiated 7-dehydrocholesterol would satisfy the "requirement".

This interpretation may explain the results of Carver and Rhian ('42). Possibly the reinforced cod liver oil used by them contained an irradiation product since concentrates of irradiated animal provitamins are used in preparing such blends. It is, of course, also a possibility that fish oils from different species or from the same species at different times may vary greatly in the efficacy for turkeys of their vitamins D.

It is more difficult to explain Hammond's ('41) results with reference cod liver oil. Since his paper was submitted for publication in August, 1940, it may be assumed that he used reference cod liver oil no. 1, the same that was used by Jukes and Sanford ('39), yet he reported good protection with a level of 80 units per 100 gm. of diet. It is well known that the calcium and phosphorus contents of the diet influence the calcification of the bones but this would not seem to resolve the difficulty since Hammond's diet, while containing more calcium and phosphorus than the diet used in these experiments, contained less of both than the diet used by Jukes and Sanford. The question arises if, by chance, the "vitamin D-free commercial concentrate" of vitamin A used by Hammond may actually have carried some vitamin D. Unfortunately, there was no group of poults in his experiment that received the basal diet without the addition of a vitamin D concentrate.

SUMMARY

1. Different levels of vitamin D from four different sources, namely, U.S.P. reference cod liver oil no. 2, a sardine oil fortified with fish liver oils, an irradiated animal sterol, and irradiated 7-dehydrocholesterol were fed to poults as supplements to a rickets-producing diet during the first 4 weeks of life. All supplements were fed on the basis of their A.O.A.C. chick unit potency.

2. There were distinct differences in efficacy among the supplements, suggesting that the poult utilizes certain forms of vitamin D with degrees of efficacy that differ from the chick and in this response exhibits a higher degree of species specificity than the chick.

3. The vitamin D of the two irradiated products was distinctly more efficacious, on the basis of chick units fed, in increasing the ash content of the bones than the vitamin D of reference cod liver oil. Comparing the results obtained over the entire range of bone ash responses, this difference in efficacy is of the order of 2 to 1. While the fortified sardine oil was measurably more efficacious than the reference cod liver oil, it gave a response curve more nearly like that of cod liver oil than like the irradiated products.

4. It is concluded that the vitamin D potency expressed in A.O.A.C. chick units is not necessarily a true measure of the value of a vitamin D supplement for turkeys.

LITERATURE CITED

BAIRD, F. D., AND D. J. GREENE 1935 The comparative vitamin D requirements of growing chicks, turkeys, and pheasants. Poultry Sci., vol. 14, p. 70.

CARVER, J. S., AND M. RHIAN 1942 The vitamin D requirements of turkey poults. Poultry Sci., vol. 21, p. 260.

HAMMOND, J. C. 1941 The vitamin D requirement of turkey poults. Poultry Sci., vol. 20, p. 204.

JUKES, T. H., AND T. D. SANFORD 1939 The vitamin D requirement of young turkeys. J. Nutrition, vol. 18, p. 71.

OFFICIAL AND TENTATIVE METHODS OF ANALYSIS 1940 5th ed., p. 371. Washington, D. C.

SCOTT, H. M., J. S. HUGHES, AND H. W. LOY 1932 Rickets in young turkeys. Poultry Sci., vol. 11, p. 177.

Reprinted from Proceedings of the
AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE
Vol. 44, 1944

Comparative Spray Deposits and Scab Control from Speed Sprayer and Single-Multiple Gun Sprayer[1]

By D. E. H. FREAR, H. J. MILLER, and F. N. FAGAN,
Pennsylvania Agricultural Experiment Station,
State College, Pa.

THE so-called Speed Sprayer, introduced during recent years as a means of reducing labor costs and speeding up the application of orchard insecticides and fungicides, has been adopted by a number of fruit growers in this section of the country. This new type of sprayer consists of a series of small, low-pressure nozzles through which the spray material is discharged, a large propeller which produces a strong air blast to drive the spray mist against the trees, and a series of vanes by which the driven mist is directed. While there is a limited amount of information available on the practical use of the Speed Sprayer, there are few references in the literature to quantitative studies on the spray deposit produced by this machine in comparison to the older conventional types of sprayers. Kadow and Hopperstead (3) have reported briefly on the deposits produced by several types of sprayers, including the Speed Sprayer. Their results indicate that considerably less spray material was deposited on the tops of the trees than on the lower portions of the trees. The greatest differences were on large trees. Hopperstead *et al.* (2), and Weber and McLean (4) have also reported briefly on the subject.

EXPERIMENTAL

During 1943, the first year of this comparative study, eight rows, each containing 18 trees, were used. Four rows were of the Stayman Winesap variety, and four, McIntosh. The rows were adjacent to each other in a nearly level 5-acre orchard block. The trees were 27 years old, the Staymans being approximately 24 feet tall and the McIntosh 30 feet.

A total of eight sprays was applied to these trees during the season of 1943. The dates of application and composition of these sprays are given in Table I. Sprays were applied simultaneously with both sprayers, two rows of each variety being sprayed with the Speed Sprayer and two with the regular high pressure apparatus. In using the latter to apply the cover sprays one man sprayed the trees from the top of a 10-foot tower, using an 8-nozzle broom, while one man covered the interior of the tree from the ground, using a single-nozzle spray gun. An attempt was made to secure thorough coverage of each tree, although the operation was done as rapidly as possible. The trees were sprayed with the broom from both sides.

With the Speed Sprayer the spray was applied from both sides of the row. The months of May and June in State College were very windy, and it was necessary to apply the first five sprays while the wind was blowing. It is to be noted that the operator of the Speed Sprayer was unfamiliar with the outfit at first. Later in the season a hood and six additional nozzles were added to the Speed Sprayer, increasing the efficiency of the machine. This was not done until the last cover spray, however, so that, in general, the results reported in this paper were secured with the machine as it came from the factory, equipped with 86 nozzles.

---

[1] Authorized for publication on December 27, 1943 as Paper No. 1215 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE I—Composition and Dates of Application of Sprays

Name and Time		Composition (Per 100 Gallons)	
Delayed dormant	April 26	Lime sulfur solution	1.5 gallons
Pre-pink	May 5	Lime sulfur solution	1.5 gallons
Full bloom	May 12	Dry lime sulfur	5.0 pounds
Petal fall	May 19	Dry lime sulfur Hydrated lime Lead arsenate Nicotine sulfate	4.0 pounds 3.0 pounds 3.0 pounds 1.0 pint
First cover	June 1	Dry lime sulfur Hydrated lime Lead arsenate Nicotine sulfate	4.0 pounds 3.0 pounds 3.0 pounds 0.5 pint
Second cover	June 14	Lead arsenate Copper sulfate Hydrated lime	3.0 pounds 1.0 pounds 3.0 pounds
Third cover	June 28	Same as second cover	
Fourth cover	July 24	Same as second cover	

Samples of 30 leaves were taken from the tops of each of six trees of each treatment, and a like number from the bottoms of the trees. Leaves were taken from the middle of the terminal growth, and were removed to the laboratory, where discs having an area (one side) of 8.3 square centimeters were cut from the leaves. These discs were counted and ashed, and lead was determined in the ash by the method of Frear and Haley (1). Each treatment thus was represented by 180 leaves from the top, and the same number from the bottom of the trees, on each sampling date. Fruit samples were taken at the date of the third cover spray, June 28, and analyzed for lead.

Counts were made of the percentage of scab-infected leaves on July 13, and records of the scab on the fruit were taken at harvest.

TABLE II—Milligrams of Lead per Square Meter of Surface Cover Sprays — Season of 1943

Date	Stayman Winesap				McIntosh			
	Top of Tree		Bottom of Tree		Top of Tree		Bottom of Tree	
	Speed Sprayer	High Pressure	Speed Sprayer	High Pressure	Speed Sprayer	High Pressure	Speed Sprayer	High Pressure
Leaves, Jun 1	2.5**	37.3	63.1**	31.2	11.7**	24.7	41.0**	27.8
Leaves, Jun 14	20.5**	60.4	81.0	70.5	13.6**	55.1	66.4**	49.6
Leaves, Jun 28	37.5**	74.8	87.6	85.6	18.9*	37.8	87.6**	68.3
Leaves, Jul 23	34.6**	69.6	71.3	68.7	18.6**	57.2	87.2**	65.2
Fruit, Jun 28	7.5	13.2	9.1	15.9	2.7**	6.7	9.1	7.6
Mean (leaves)	23.8	60.5	75.8	64.0	15.7	43.7	70.6	52.7
Per cent scab, leaves, Jul 13	—	—	—	—	6.9**	2.3	0.3	0.3

*Significant difference between two figures. (Speed Sprayer vs. High Pressure) odds 19:1.
**Significant difference between two figures. (Speed Sprayer vs. High Pressure) odds 99:1.

Results

Spray Deposits:—The results of the lead analyses are given in Table II, expressed as milligrams of lead per square meter of surface. The high pressure sprayer produced a significantly higher deposit on the leaves at the top of the trees than did the Speed Sprayer. This was true throughout the season, and on both varieties of apples. On the lower leaves, the Speed Sprayer produced the heavier deposit in five out of eight cases. The McIntosh trees showed a consistently heavier deposit on the lower leaves when sprayed with the Speed Sprayer than with the high pressure outfit.

TABLE III—Amounts of Spray Mixture Required to Cover Each Tree

Date	Average Number Gallons Per Tree	
	Speed Sprayer	High Pressure
Apr 26	15.1	13.0
May 5	13.9	12.3
May 12	13.8	11.6
May 19	15.7	13.8
Jun 1	17.5	17.0
Jun 14	21.1	18.8
Jun 28	20.8	17.4
Jul 24	23.0	18.8
Mean	17.6	15.3

The results of the analyses of the fruits were not so conclusive, only one significant difference being observed, in the case of the McIntosh top fruits, where the deposit was heavier with the high pressure sprayer. The mean values for all sampling dates are shown in Table II, and indicate quite clearly that the high pressure sprayer produced a greater spray deposit in the tops of the trees, while the Speed Sprayer produced the heavier deposit in the bottoms of the trees.

Amounts of Spray Required:—Table III shows the amounts of spray required to cover each tree with the two types of sprayers. In every case a greater number of gallons was required to cover each tree with the Speed Sprayer, the mean difference being 2.3 gallons per tree.

Effect of Type of Sprayer on Disease Control:—The scab counts on leaves taken on July 13, revealed no infection on the Stayman variety. On the McIntosh, however, there was a significant difference in the percentages of the amount of scab in the tops of the trees. The leaves from the tops of the trees sprayed with the high pressure sprayer, and which (Table II) had a heavier spray deposit, had significantly less scab than those from the trees sprayed with the Speed Syrayer. There was no significant difference in the leaves from the bottoms of the trees.

At the time of the harvest, 600 fruit were examined for apple scab, 300 each from the top and bottom of the McIntosh trees. Top fruits sprayed with the Speed Sprayer showed 16.1 per cent scab, and 7.8 per cent when sprayed with the high pressure outfit. The corresponding percentages on fruits from the bottoms of the McIntosh trees were 1.5 and 0.9, respectively. There was no scab on the fruit of the Stayman trees, and no codling moth injury on any fruit of either variety.

Grading records of 535 bushels of McIntosh and 473 bushels of Stayman fruits are given in Table IV. Two composite samples of McIntosh culls, one from each spray treatment, were examined individually to determine the cause of rejection. The percentages of the original crop rejected because of scab and mechanical injury were calculated from this detailed examination, and are also included in Table IV. The proportion of fruits rejected in this commercial grading operation because of scab agrees closely with the averages of the random fruit counts given in the preceding paragraph. With both varieties, the percentages of packing house culls were lower, and the percentage of commercial packed fruit higher, when the trees were sprayed with the high pressure sprayer.

TABLE IV—Results of Commercial Grading of Apple Crop from Two Varieties Sprayed with the High Pressure and Speed Sprayer

	Stayman		McIntosh	
	Speed Sprayer	High Pressure	Speed Sprayer	High Pressure
Bushels graded	301	172	215	320
Ciders* (per cent)	1.3	1.7	8.8	5.6
Packing house culls (per cent)	17.6	11.6	27.4	20.0
Mechanical injuries (per cent)	—	—	(14.9)	(11.9)
Scab (per cent)	—	—	(9.6)	(4.8)
Commercial pack (per cent)	81.1	86.7	63.8	74.4

*Under 2¼ inches; not sorted except for size.

Time and Labor Factor:—The 500-gallon Speed Sprayer was hauled through the orchard by a track tractor. The tractor operator also operated the sprayer. The sprayer was refilled in the orchard by a truck mounted supply tank. Agitation power and refilling pump for the supply tank were operated by power takeoff from the supply truck engine. The supply tank operator mixed the spray material as the tank was being filled with water at the loading station. These two men operating truck and sprayer are classed as a spray unit.

The high pressure sprayer was also drawn by a track tractor, but the tractor operator did no spraying. For the first four sprays one man applied the sprays, while in the four cover sprays two men were used, one on the ground and one on a tower attached to the sprayer. The high pressure outfit was not serviced in the orchard by a supply tank but the entire crew returned to the filling station for refilling and spray mixing.

The Speed Sprayer unit averaged in the first four sprays 2.36 times as fast as was the spraying done by the two men operating the 35 gallon high pressure sprayer of 400 gallons capacity. In the four cover sprays, the Speed Sprayer unit averaged 2.48 times as fast with a two-man crew as did the high pressure sprayer unit with a three-man crew.

CONCLUSIONS

The Speed Sprayer, as used in orchard trials on two varieties of apples during the 1943 season, was compared with the conventional high pressure sprayer. Deposits of spray produced by the Speed Sprayer were lower on the tops of the trees and higher on the bottoms of the trees than those from the high pressure sprayer. The low spray deposit on the tops was reflected in increased scab incidence on the leaves and fruits of the McIntosh variety, and the amount of commercial packed fruit was lower from the trees sprayed with the Speed Sprayer. The name "Speed Sprayer" indicates rapid application of material. The study of time and labor in this test shows applications were made on an average of more than twice as fast with the same amount of labor as by the high pressure sprayer. These studies will be continued during the 1944 season.

LITERATURE CITED

1. Frear, D. E. H., and Haley, D. E. A simplified method for the rapid determination of lead residues on apples. *Pa. Agr. Exp. Sta. Bul.* 304. 1934.
2. Hopperstead, S. L., Goodwin, M. W., and Rice, P. L. Spraying efficiency of the new types of spray equipment. *Phytopath.* 33: 1114. 1943.
3. Kadow, K. J., and Hopperstead, S. L. An evaluation of new spray equipment. *Trans. Penins. Hort. Soc.* 32: 13–18. 1942.
4. Weber, A. L., and McLean, H. C. Spray coverage of apple trees as affected by different methods of application. *Proc. Amer. Soc. Hort. Sci.* 42: 285–288. 1943.

SUN HELME

(Continued from page

result is a stiff laminated paper shell drawn to the exact shape of the master mold and in a standard head size.

The next step is the application of a thin coating of cork both inside and outside this shell. First, the inside of the shell is sprayed with "Plaskon" and then filled to the brim with powdered cork which is lightly depressed by hand and then emptied into another shell. Enough of the cork powder adheres to the adhesive to form a uniform coating over the entire surface. By a similar method, the exterior of the shell receives its layer of cork powder, after which the shell is re-inserted in the hydraulic mold and the new surfaces compressed and bonded.

Back to the spray booth once more and the shell is Plaskon-sprayed on the outside only, then placed on the master mold and a layer of helmet cloth applied. The cloth has already been cut into squares and one square is stretched over each shell and heat and pressure applied to complete the bond. The combination of plastic adhesive, heat and pressure does its job swiftly and permanently.

At the trimming bench, a small saw cuts away superfluous helmet cloth and paper shell, leaving a deep unfinished brim, the inside of which is next painted with a thermoplastic. Heat and pressure treatment in still another hydraulic press rolls this painted edge and forms a strong secure brim of uniform size.

At this stage, preliminary inspection rejects any helmets that may be faulty in finish or appearance. Approved helmets pass on to a machine which punches eighteen holes around the crown to accommodate the leather sweat band, chin strap and the cord by which the helmet is adjusted to an individual's head size. Meanwhile, separate operations have produced the components for the complete "harness" assembly which is now fitted within the helmet shell.

Now, here is your helmet ready for final inspection before being wrapped in tissue paper and packed in shipping cartons for delivery. For military service helmets are required to pass a water test which specifies that twenty-four hour immersion in water shall leave no signs of disintegration at the end of that period.

Ready for active service, the modern sun helmet provides the Canadian Soldier with a cool head for warm weather warfare. So, soldier, spare that tree! It may one day

the

the

Shell

St

FANWEED SOURCE

JOH

Northern
United St

HOWA
Pr
Pen
Sta

As the result of thors it has been plant contain a hi of such quality that as the major veg i.e., those from c beans and peanuts certain unusual pr in the preparation gines, pneumatic to ration of rubber su and similar compo suitable for use in in the manufacture terproof materials. oil constituent and margarine or made

Fanweed seed oi oil which the Unite quantities from Ja

Among the most vestigation is that high percentage of and can be produce

Test plantings a shown that on irrig proximately 36 bus would mean a yie acre for each crop so that two crops a of 1000 pounds of of any oil-produci which does not gro

Furthermore, it the meal obtained seeds with oil solve rich in nitrogen (3 dry extracted meal) ment the extracted tilizer.

It is also of int contains fibers com Further investigatio fibers are of value fields.

Description

Fanweed, along of the botanical gr

to the writers' investigations very little was known about the oil obtainable from fanweed seeds. A few years ago the Bureau of Plant Industry of the United States Department of Agriculture made a study of the oil-content of several weeds commonly found in grain

SUN HELMETS

(Continued from page 157)

result is a stiff laminated paper shell drawn to the exact shape of the master mold and in a standard head size.

The next step is the application of a thin coating of cork both inside and outside this shell. First, the inside of the shell is sprayed with "Plaskon" and then filled to the brim with powdered cork which is lightly depressed by hand and then emptied into another shell. Enough of the cork powder adheres to the adhesive to form a uniform coating over the entire surface. By a similar method, the exterior of the shell receives its layer of cork powder, after which the shell is re-inserted in the hydraulic mold and the new surfaces compressed and bonded.

Back to the spray booth once more and the shell is Plaskon-sprayed on the outside only, then placed on the master mold and a layer of helmet cloth applied. The cloth has already been cut into squares and one square is stretched over each shell and heat and pressure applied to complete the bond. The combination of plastic adhesive, heat and pressure does its job swiftly and permanently.

At the trimming bench, a small saw cuts away superfluous helmet cloth and paper shell, leaving a deep unfinished brim, the inside of which is next painted with a thermoplastic. Heat and pressure treatment in still another hydraulic press rolls this painted edge and forms a strong secure brim of uniform size.

At this stage, preliminary inspection rejects any helmets that may be faulty in finish or appearance. Approved helmets pass on to a machine which punches eighteen holes around the crown to accommodate the leather sweat band, chin strap and the cord by which the helmet is adjusted to an individual's head size. Meanwhile, separate operations have produced the components for the complete "harness" assembly which is now fitted within the helmet shell.

Now, here is your helmet ready for final inspection before being wrapped in tissue paper and packed in shipping cartons for delivery. For military service helmets are required to pass a water test which specifies that twenty-four hour immersion in water shall leave no signs of disintegration at the end of that period.

Ready for active service, the modern sun helmet provides the Canadian Soldier with a cool head for warm weather warfare. So, soldier, spare that tree! It may one day be the mother of a large family of sun helmets; the proud father, chemistry.—*(Note: Reprinted from the C-I-L Oval, a Magazine of Industrial Chemistry, published by Canadian Industries Limited, in Montreal, Canada.)*

Shells sprayed with heat setting resin, dry cork powder poured in and out, air-dried, hydraulic pressed. 5 tons.

Stretching the khaki cloth on the resin treated shells.

The finished army sun helmet. Weight 6½ oz., very strong and waterproof.

WASHINGTON STATE FARM CHEMURGIC COMMITTEE

At the annual meeting of the Washington State Farm Chemurgic Committee, Dr. H. K. Benson was re-elected chairman; Mr. L. E. Lowe, former secretary, was elected vice-chairman; Mr. D. B. Noble, agricultural agent for the Milwaukee Railroad, Spokane, was elected secretary. Mr. D. B. Leonard, former vice-chairman will be active in the development of special project work.

FANWEED, A POTENTIAL MAJOR SOURCE OF VEGETABLE OIL

JOHN R. CLOPTON, Ph.D.
Research Chemist
Northern Regional Research Laboratory
United States Department of Agriculture
Peoria, Illinois
and
HOWARD O. TRIEBOLD, Ph.D.
Professor of Biochemistry
Pennsylvania State College
State College, Pennsylvania

As the result of investigations carried out by the authors it has been shown that the seeds of the fanweed plant contain a high grade vegetable oil. This oil is of such quality that it can be used for the same purpose as the major vegetable oils produced commercially, i.e., those from cottonseed, flaxseed, corn germ, soybeans and peanuts. In addition, it is known to have certain unusual properties making it suitable for use in the preparation of special lubricants for marine engines, pneumatic tools and other machinery, the preparation of rubber substitutes, rubber extenders (factices) and similar compounds. Its drying properties make it suitable for use in paints, varnishes and lacquers, and in the manufacture of linoleum, oilcloth and other waterproof materials. The purified oil is a suitable salad oil constituent and may be hydrogenated for use in margarine or made into soaps, greases, etc.

Fanweed seed oil is an ideal substitute for rapeseed oil which the United States formerly imported in large quantities from Japan.

Among the most interesting facts revealed by this investigation is that fanweed seeds contain an unusually high percentage of oil (30 to 40 percent by weight) and can be produced readily in unlimited quantities.

Test plantings at the Montana State College have shown that on irrigated land 1500 pounds of seed (approximately 36 bushels) per acre may be obtained. This would mean a yield of about 500 pounds of oil per acre for each crop. The plant matures early enough so that two crops a season are easily possible. A yield of 1000 pounds of oil per acre would far exceed that of any oil-producing crop except possibly the peanut which does not grow in the same areas as fanweed.

Furthermore, it has been found by the writers that the meal obtained by grinding and extracting fanweed seeds with oil solvents, such as petroleum ether, is very rich in nitrogen (33 percent protein on the basis of the dry extracted meal). This suggests that by proper treatment the extracted meal could be used as a feed or fertilizer.

It is also of interest to find that the fanweed plant contains fibers comparable to those of the flax plant. Further investigations in progress may show that these fibers are of value in the textile industries and related fields.

Description of the Plant and Its Habits

Fanweed, along with rape and mustard, is a member of the botanical group, Cruciferae, which is commonly known as the mustard family. Botanically, fanweed is known as *Thlaspi arvense*. It is known by other common names, such as Frenchweed, field penny cress, bastard cress, Irish mustard, mithridate mustard, etc. The name, fanweed, was given to it because of the characteristic, fanlike shape of the seed pods. In the Canadian Provinces it is generally referred to as stinkweed. This is because of the rather unpleasant odor produced when the plant tissues are bruised. In the Red River Valley and adjoining regions of Minnesota and North and South Dakota it is commonly called Frenchweed, while farther west fanweed is the most popular name.

Fanweed grows abundantly in most of the northwestern and North-central United States and is common in the central states west of the Mississippi River. It also grows extensively in Canada, in east-central Russia and to a limited extent in most of the European countries. It is believed to have been introduced into this country from Europe.

Fanweed is an erect annual which under favorable conditions of growth attains a height of from one to two feet. The leaves are somewhat oblong, narrow shaped and toothed. The flowers are white, have four petals and are only about one-fourth inch in diameter. Each flower in a terminal raceme gives place, on ripening, to flattened, fan-shaped pods which are about one-half inch in diameter, broadly winged and have a deep notch at the top. The pods have two lobes, each of which contains four to eight small, oval-shaped reddish brown seeds. The seeds average 1.5 mm. in width, 2 mm. in length and 1 mm. in thickness. The seed covering is rather hard and is deeply creased with concentric ridges.

In areas inhabited by fanweed it is considered a troublesome weed. When eaten by dairy animals, a garlic-like taste is imparted to the milk, cream and butter. This is due to the presence of traces of oil of garlic (allyl sulfide) and oil of mustard (allyl isothiocyanate) set free when the seeds become moist. These volatile principles are held in an odorless and inactive form (constituents of glycosides) in the dry seeds. There are enzymes present in the seeds which can hydrolyze the glycosides and free the volatile oils, but this can occur only when the seeds become wetted.

The fanweed crop would require a minimum of care. It can be sown by drill in shallow-cultivated soil, and harvested with the usual farm equipment required for the cereal crops. Though the best yields may be obtained from irrigated land, the plant thrives very well on semi-arid and arid land.

Fanweed seeds can be flaked and extracted with equipment commonly used on other oil-bearing seeds such as the soybean. The extracted oil has a greenish-yellow color, similar to most extracted seed fats which upon decolorization by the usual methods leaves a pale yellow oil resembling other refined vegetable oils in odor and flavor.

No time-consuming development program is necessary to bring about the mass production of fanweed seed oil. Quantities of seed can be harvested from idle land in the fanweed areas this coming season if only the farmers can be assured of a market for this crop.

Nothing of importance has been done in the United States to develop uses for products of the fanweed plant. A review of the literature indicates that previous to the writers' investigations very little was known about the oil obtainable from fanweed seeds. A few years ago the Bureau of Plant Industry of the United States Department of Agriculture made a study of the oil-content of several weeds commonly found in grain screenings. It was reported that fanweed seeds contain 33.7 percent oil by weight and suggested that this oil might be used as a lubricant. However, there were no further developments.

Although fanweed is just as old botanically as the different species of rape, strangely enough, its oil-producing possibilities have been almost completely overlooked, while varieties of rape plants have been cultivated as an oil-seed crop for centuries.

As well as being one of the major foods, oils and fats play a particularly dominant role as an industrial raw material. This is especially true in time of war. In view of our present shortage of fats and oils it is indeed timely that other potential sources of these materials be found. It seems certain that the fanweed plant has possibilities of becoming one of the major oil-producing crops in the United States, as well as in Canada.

The authors have made the first complete study of composition and properties of fanweed seed oil. A report on this work will appear soon in *The Chemurgic Digest*. This will be followed by further reports on the possible industrial uses of this oil.

References

Clopton, John R., "A Study of the Composition and Properties of Fanweed Seed Oil With the View of Finding a Substitute For Rapeseed Oil." Ph. D. Thesis, Pennsylvania State College, May 15, 1943.

Clopton, John R. and Triebold, Howard O., "Fanweed Seed Oil, Potential Substitute for Rapeseed Oil," Ind. Eng. Chem., 36:218-19 (1944).

ALCOHOL PRODUCTION REPORT

Production of 190-proof alcohol in the first four months of the year was at a record rate of 192,200,000 gallons, as compared with a production of 137,400,000 gallons during the first four months of 1943, members of the War Production Board's Industrial Alcohol Producers Industry Advisory Committee were informed in June, WPB reported.

The total 1944 alcohol supply is now estimated at 612,400,000 gallons of 190-proof alcohol, against an estimated requirement of 633,900,000 gallons, according to WPB's Chemicals Bureau.

Dr. Walter G. Whitman, assistant director of the Chemicals Bureau, informed the committee that preliminary estimates of supply and requirements for 1945 indicated requirements would be 634,000,000 gallons against an estimated supply of 639,800,000 gallons. The revised estimates of requirements in terms of millions of gallons of 190-proof alcohol are as follows:

	1942	1943	1944	1945
Synthetic rubber		126.0	365.0	365.0
Direct military and Lend-Lease	71.2	102.0	78.6	89.0
Indirect military and civilian	128.0	147.9	160.3	160.0
Anti-freeze	30.0	50.8	30.0	20.0
	229.2	427.6	633.9	634.0

Members of the committee were informed by Chemicals Bureau officials that the alcohol-from-potato program, which was established to provide an outlet for surplus potatoes from the 1943 crop, was about completed. They said that 300,000 gallons of alcohol had been distilled from 3,200 tons of dehydrated potatoes, which had been obtained from 19,200 tons of raw potatoes.

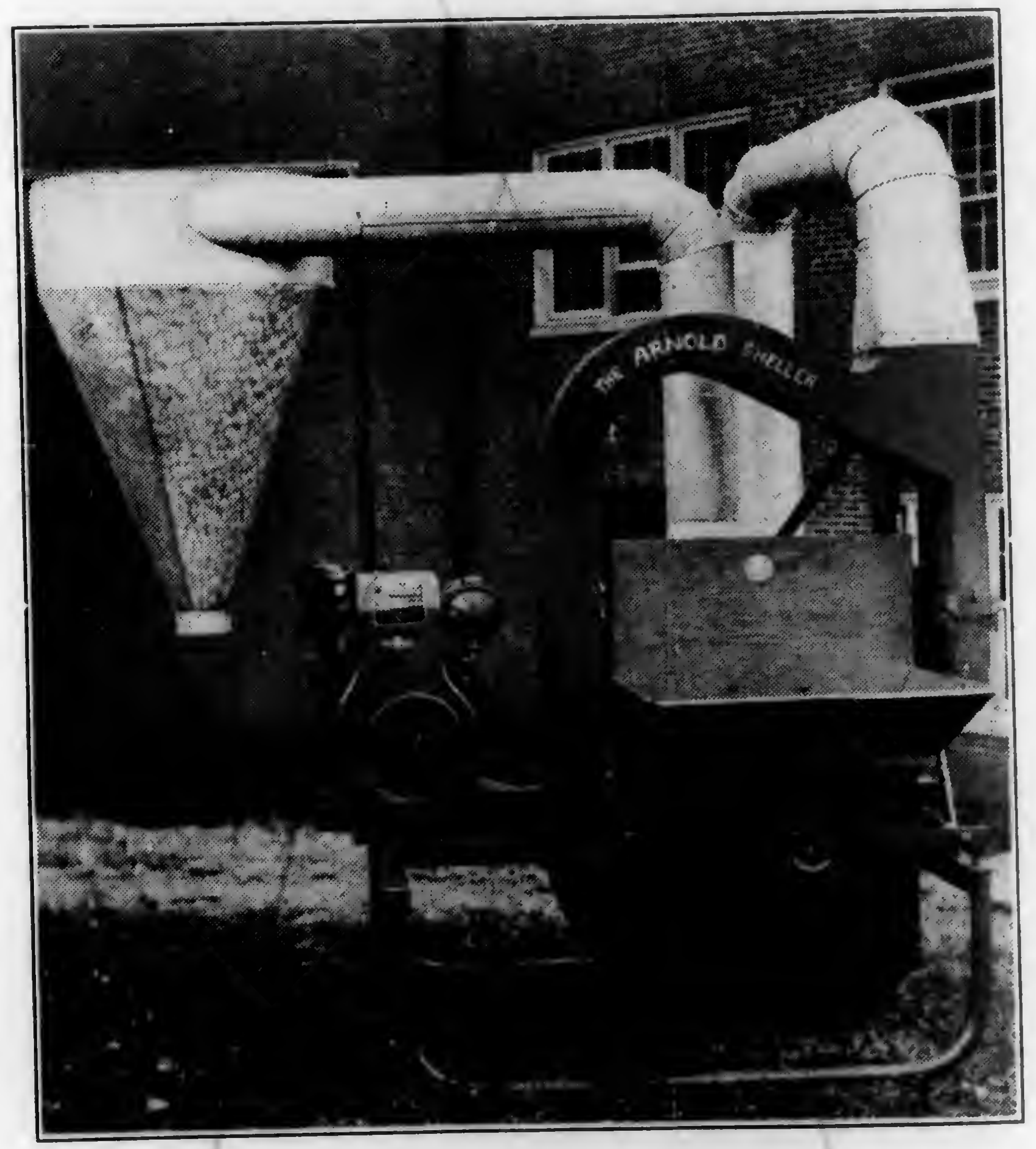
Asked to advise on the use of additional quantities of rye in their production (some distilleries have been using rye flour on an experimental basis) members of the committee reported that additional quantities of rye could not be used without reducing production. The rye flour, they said, is difficult to handle, gums up their facilities and increases manpower costs.

Members of the committee told Government officials that they felt the minimum safe stockpile figure, estimated earlier at 100,000,000 gallons, could now be revised downward. The present stockpile is 86,100,000 gallons.

PUBLICATIONS OF CHEMURGIC INTEREST

"The Improved Castor-Bean Sheller. Description, Operation, and Adjustment of the 24-inch Sheller and Separator." By H. A. Arnold and M. A. Sharp. *Bulletin No. 187*, The University of Tennessee, Agricultural Experiment Station, Knoxville, Tennessee, January, 1944.

This bulletin can be secured by writing to the University of Tennessee at the above address.



Side view of improved castorbean sheller.

FISH-OIL VITAMINS COMBINED

Looking for high-vitamin oils to supplement supplies of cod-liver and other fish oils which have been reduced by wartime conditions, two agricultural experiment stations have been studying extraction of fish oils from species of fish not commonly used. On the West Coast, the Oregon agricultural experiment station has worked with various species. On the Atlantic Coast, the Florida agricultural experiment station found that shark-liver oil was an excellent substitute for cod-liver oil. Since cod-liver oil is high in vitamin D and relatively low in vitamin A, while in shark-liver oil the reverse is true, the investigators suggest a combination of the two oils.

ALCOHOL FROM WASTE SULPHITE LIQUOR

By M. M. ROSTEN

(Continued from last issue)

Alcohol Quality

Average quality data for ethyl alcohol from closed receiver for the months of October and November, 1943 are summarized in Table IV:

TABLE IV

	Average of daily tests		
	Oct. 1943	Nov. 1943	Average
Residual acidity	0.0021%	0.0029%	0.0025%
Permanganate time	36 min.	28 min.	32 min.
Aldehydes, parts per 100,000 ..	1.2	1.4	1.3
Fusel oil, parts per 100,000....	7.6	8.7	8.2
Esters, parts per 100,000....	0.36	0.55	0.46
Methyl alcohol ..	each day less than 0.1%	each day less than 0.1%	each day less than 0.1%
	(0.1% is the minimum determinable quantity by regular test)		
Proof, (Canadian)	68.8 O.P.	68.7 O.P.	68.8 O.P.
Residue on evaporation ..	0.0016%*	0.0018%*	0.0017%*

* Average test on shipments made, not daily average.

The Company is under instructions to produce ethyl alcohol up to United States Army specifications No. 1 and the alcohol complies with and in some tests substantially exceeds these specifications. The quality given in Table IV must not be regarded in any sense as a limiting quality. As in the case of any other industrially produced alcohol, the quality of the product depends on distillation equipment and operation. As in the case of any other industrially produced alcohol, granted the equipment and its proper operation, alcohol can be produced to meet any given industrial specifications.

The residual acidity is low and averages only one-quarter of the maximum permitted by specifications. The permanganate time is indicative of a very good grade industrial alcohol. Occasional individual days have tested 45 minutes. Aldehydes are low in line with the pergamanate time. The data given for fusel oil are based on colorimetric test using our own fusel oil as a standard. Esters are low. Methyl alcohol is always below the limit of determination. Residue on evaporation averages less than one-third the maximum allowable amount. The proof strength is high (68.8 O.P. corresponds to 96.3 percent EtOH by volume).

One question on quality frequently asked is how much sulphur the alcohol contains. This question arises because sulphite liquor is the raw material and may be an honest inquiry or a blatant canard which may take the form of dubbing the product "sulphite alcohol." From the standpoint of the industry it must be answered without the slightest evasion. The sulphur content of Thorold alcohol as independently analyzed by a reputable laboratory, has tested between 1.4 and 2.1 parts per million total sulphur. These analyses are on the product as presently manufactured and not on specially selected samples. Such concentration is pretty close to the limit of determination.

It would be interesting to note that Les Usines de Melle azeotropic method of distillation of waste sulphite liquor beer could deliver absolute water-free ethyl alcohol, and pure methanol, using only about 100 pounds of steam at 10 pounds pressure per gallon of alcohol. Practically no power is required for distilling process. Sulphur dioxide is recovered during distillation. Alcohol received is sulphur-free.

Methyl Alcohol Fraction

The "methyl alcohol fraction" includes the methyl alcohol formed in the digester, which comes through the system admixed with heads impurities removed during distillation. The methyl alcohol fraction draw is thus really a concentrate of impurities from the main product. As such, its regulation is a principal means of operating quality control of the ethyl alcohol. Because of its small quantity (approximately 5 percent of the total distillate), and since it is a distilled concentrate of aldehydes, etc., its analysis is particularly sensitive to quality changes, and is watched with this in view. There is many-fold the quality variation in this fraction than there is in the ethyl alcohol. Production policy is, of course, to maintain a proper quality of ethyl alcohol and let the methyl alcohol fraction act as a shock absorber.

Average daily test data for the methyl alcohol fraction for October and November, 1943 are given in Table V.

TABLE V—METHYL ALCOHOL FRACTION

	Average of daily tests		
	Oct. 1943	Nov. 1943	Average
Residual acidity..	0.22	0.13	0.18
Aldehydes. parts per 100,000....	2000	1850	1925
Methyl alcohol ..	83.9%	81.2%	82.6%
Proof (Canadian)	70.6 O.P.	69.5 O.P.	70.1 O.P.

The concentration of impurities, for example of aldehydes of the order of 1500 times, of residual acidity 100 times that in the ethyl alcohol, will be apparent.

By an additional distillation process (not installed at Thorold) it is possible to obtain pure methyl alcohol from this fraction, if desired.

Fusel Oil

Fusel oils are removed from the rectifying column and amount to 0.2 percent of the total distillate. This product is also under allocation.

Conversion Cost

In Table VI the conversion costs are given based on the Thorold plant quantities and American prices on one gallon of alcohol in a small and average size pulp mill. Overhead and amortization charges are not included. In a large pulp mill the conversion cost could be estimated 8 to 10 cents per gallon of alcohol.

282

[Reprinted from FOOD RESEARCH, 1944, Vol. 9, No. 5, Pages 415-417]

VITAMIN CONTENT OF SOME TROPICAL FRUITS, THEIR JUICES AND NECTARS

VICTOR L. QUINONES, N. B. GUERRANT, AND R. ADAMS DUTCHER
Department of Agricultural and Biological Chemistry, Pennsylvania State College, State College, Pennsylvania

(Received for publication, February 2, 1944)

The present report deals with the determination of carotene, thiamin, riboflavin, and ascorbic acid in certain tropical and subtropical fruits. These studies include guavas, papayas, and mangoes obtained from the Florida Subtropical Experiment Station.[1] Lemons, oranges, and grapefruit were purchased on the local market. Canned orange juice, grapefruit juice, vegetable juice cocktails, tomato juice, and apricot nectar were also purchased on the open market. Nectars of guava, mango, tamarind, banana, quenepa, papaya, and soursap and pineapple juice were prepared by the Division of Investigation and Development of the Department of Agriculture of Puerto Rico or by Puerto Rican commercial concerns.

EXPERIMENTAL PROCEDURE

Chemical methods were employed for vitamin assay. Carotene was determined by extraction of the pigments with petroleum ether after saponification with 10-per cent alcoholic NaOH, chromatographic separation of pigments in the petroleum ether extract by means of a dicalcium phosphate column, and measuring the carotene against pure β carotene as a reference standard in a Cenco-Sheard spectrophotelometer.

Thiamin was determined by the thiochrome method of Hennessy and Cerecedo (1939), using the Pfaltz and Bauer fluorometer. Riboflavin was also measured fluorometrically according to the method of Conner and Straub (1941). Ascorbic acid was estimated by direct titration with 2,6-dichlorobenzeneoneindophenol as originated by Tillmans and modified by Bessey and King (1933), except that extraction was performed in a Waring blender with trichloroacetic and metaphosphoric acids.

A summary of results[2] obtained on four tropical fruits and pineapple juice for carotene, thiamin, riboflavin, and ascorbic acid is given (Table 1); the data obtained on the ascorbic acid content of nectars and juices is shown (Table 2).

DISCUSSION

Owing to war conditions it was impossible to study varieties, stages of ripeness, etc., as originally planned. The mangoes were all of the Haden

---

[1] We wish to acknowledge the helpful co-operation of Dr. G. D. Ruehle who furnished some of the subtropical fruits used in these studies.

[2] Since the above data were submitted for publication the following subtropical fruits were received from Dr. Philip J. Westgate of the Subtropical Experiment Station, Homestead, Florida, and were found to contain the following amounts of ascorbic acid: (1) carambola, 35.3 milligrams per 100 grams fresh fruit; (2) carissa, 6.48 milligrams per 100 grams fresh fruit; (3) loquats (overripe), 3.96 milligrams per 100 grams fresh fruit.

TABLE 1

Vitamin Content of Some Tropical Fruits

Fruits	Condition of fruit	Carotene	Thiamin	Riboflavin	Ascorbic acid
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Papaya No. 1	Fairly ripe	......	......	......	43
Papaya No. 2	Fairly ripe	.903	.042	.050	29
Papaya No. 3	Fairly ripe	.790	.042	.038	32
Papaya No. 4	Ripe	.990	.045	.038	63
Papaya No. 5	Somewhat green	.713	.039	.052	40
Guava No. 1	Ripe and hard	.307	.042	.027	246
Guava No. 2	Overripe, soft	.430	.039	.032	190
Guava No. 3	Ripe and hard	.025	.045	.017	296
Guava No. 4	Overripe, soft	.014	.042	.027	196
Orange No. 1	Ripe	.109	.117	.028	35
Orange No. 2	Ripe	.118	.105	.034	40
Orange No. 3	Ripe	.127	.117	.024	44
Mango No. 1	Ripe	.506	.060	.073	17
Mango No. 2	Ripe	.505	.063	.037	15
Mango No. 3	Ripe	.527	.057	.050	17
Pineapple juice No. 1	From cull fruits	.023	.051	.017	21
Pineapple juice No. 2	From cull fruits	.026	.051	.023	21

TABLE 2

Ascorbic Acid Content of Various Nectars and Juices as Determined by Dye-Titration Method

Sample of material	Description	Ascorbic acid (vitamin C)
		mg./ 100 ml.
Pineapple juice	Canned from cull pineapples in Puerto Rico (1941)	22
Papaya nectar	Canned (Puerto Rico, 1941)	16
Guava nectar	Canned (Puerto Rico, 1941)	51
Tamarind nectar	Canned (Puerto Rico, 1941)	5
Mango nectar	Canned (Puerto Rico, 1941)	20
Banana nectar	Canned (Puerto Rico, 1941)	3
Soursap nectar	Canned (Puerto Rico, 1941)	11
Quenepa nectar	Canned (Puerto Rico, 1941)	2
Grapefruit juice	Canned (local market)	44
Orange juice	Canned (local market)	40
Tomato juice	Canned (local market)	22
Vegetable juice cocktail No. 1	Canned (local market)	7
Vegetable juice cocktail No. 2	Canned (local market)	10
Apricot nectar	Canned (local market)	3
Lemon juice	Fresh (local fruit market)	36
Orange juice	Fresh (local fruit market)	53
Grapefruit juice	Fresh (local fruit market)	39

variety and were at practically the same stage of ripeness. Consequently, no marked differences are noted in vitamin content. The pineapple juices were known to be made from culls which had ripened in the Puerto Rican warehouse and are probably not comparable with high-grade pineapple juice.

The carotene content of papayas tends to increase with ripening. There was no uniformity in the carotene content of guavas, of which there were two varieties, and regarding which no information was available. One variety of guava is represented by Samples 1 and 2 and the other by Samples 3 and 4 (Table 1). Apparently, the ascorbic acid content of guavas tends to decrease as they become overripe. It would appear that this fruit is an excellent source of ascorbic acid, being superior to oranges in this respect.

SUMMARY

The carotene, thiamin, riboflavin, and ascorbic acid content of papayas, guavas, mangoes, oranges, and commercially canned pineapple juice have been determined by chemical methods. The ascorbic acid content of a number of fruit juices and fruit nectars have also been studied. The limited data seem to indicate that papayas are an excellent source of carotene and a good source of ascorbic acid but are less rich in thiamin and riboflavin than the other fruits.

Guavas are excellent sources of ascorbic acid while carotene seems to be the principal factor contributed by the mango. Guava nectar compares very favorably with orange juice and lemon juice as a source of ascorbic acid.

REFERENCES

HENNESSY, D. J., AND CERECEDO, L. R., 1939. The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. **61**, 179-183.

CONNER, R. T., AND STRAUB, G. J., 1941. Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem., Anal. Ed. **13**, 380-388.

BESSEY, O. A., AND KING, C. G., 1933. The distribution of vitamin C in plant and animal tissue and its determination. J. Biol. Chem. **103**, 687-698.

Reprinted from Analytical Edition, INDUSTRIAL AND ENGINEERING CHEMIST
Copyright 1944 by the American Chemical Society and reprinted by permis

Physical Constants of Methyl Esters of C Fatty Acids

VAPOR PRESSURE

PAUL M. ALTHOUSE AND HOWARD O. TRIEB
Department of Agricultural and Biological Chemistry, The Pennsylvania State

Methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linolic acids were obtained in a pure state by repeated fractional distillation. Vapor pressure curves and decomposition pressures and temperatures have been determined for each of the methyl esters by the method described by Ramsay and Young. With the exception of the C_{18} series, it has been shown that an ester fraction can be identified and its purity ascertained by means of its vapor pressure curve. With the aid of the decomposition data, it is possible to eliminate excess decomposition by controlling the pressure and hence the boiling temperature of fractional distillation.

FRACTIONAL distillation is recognized as an excellent method for separating and purifying the methyl esters of the naturally occurring fatty acids. The fractions obtained from such a distill
fractions,
Two
such
an
i

find the boiling point
pressure. This last
separation and purifi
It is also possible to
the temperature and
This information can
tional distillation of

PREPAR

The methyl esters
palmitic, stearic, and
repeated fractional
heated fractionating
and Lux (2), fitted
head. The purity o
chemical analyses, c
ber, and thiocyanoge

Figure 2. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

---

Figure 1. Va

since in the ma
sition occurs far
I gives the app
sures and tem
methyl esters s
boiling points f
different pressu

From the dat
conclusions may
With the e
whose vapor p
Figure 2 are pra
to identify a pu
its vapor press
information av
to find the boil
at several diff
superimposing
one of the abov
the ester. Suc
complished in
considerable de

Reprinted from Analytical Edition, INDUSTRIAL AND ENGINEERING CHEMISTRY, Vol. 16, Page 605, October 15, 1944

Copyright 1944 by the American Chemical Society and reprinted by permission of the copyright owner

Physical Constants of Methyl Esters of Commonly Occurring Fatty Acids

VAPOR PRESSURE

PAUL M. ALTHOUSE AND HOWARD O. TRIEBOLD

Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

Methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linolic acids were obtained in a pure state by repeated fractional distillation. Vapor pressure curves and decomposition pressures and temperatures have been determined for each of the methyl esters by the method described by Ramsay and Young. With the exception of the C_{18} series, it has been shown that an ester fraction can be identified and its purity ascertained by means of its vapor pressure curve. With the aid of the decomposition data, it is possible to eliminate excess decomposition by controlling the pressure and hence the boiling temperature of fractional distillation.

FRACTIONAL distillation is recognized as an excellent method for separating and purifying the methyl esters of the naturally occurring fatty acids. The fractions obtained from such a distillation may be divided into two classes: the pure ester fractions, and the mixed fractions containing two or more esters.

Two types of analyses are possible for the identification of such fractions. The best and most commonly used are chemical analyses which involve the determination of such values as the iodine number, neutral equivalent, and thiocyanogen number. These procedures are difficult and time-consuming, and are impractical when the fractions are very small. Accordingly, it seemed advisable to investigate the second possibility, that of substituting physical measurements for the usual chemical methods. Consequently, the problem resolved itself into a search for suitable physical constants which would yield information as to the purity and quantity of any ester in a given fraction.

Vapor pressure was the first physical constant investigated. By means of this determination the purity of a substance may be ascertained in a very short time and with a considerable degree of accuracy. By choosing a dynamic method, it is also possible to find the boiling point of the substance in question at any given pressure. This last fact in itself is of great importance in the separation and purification of materials by fractional distillation. It is also possible to learn from a vapor pressure determination the temperature and pressure at which decomposition occurs. This information can be utilized to great advantage in the fractional distillation of organic compounds.

PREPARATION OF THE METHYL ESTERS

The methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic acids were prepared and purified by repeated fractional distillation through a 16-plate, electrically heated fractionating column, of the type described by Whitmore and Lux (*2*), fitted with a total reflux, partial take-off distilling head. The purity of each ester was determined by the usual chemical analyses, consisting of neutral equivalent, iodine number, and thiocyanogen number. The methyl ester of linolic acid was purified first, by repeated crystallization of the tetrabromo derivative, and then, after regeneration, by repeated fractional distillations. These pure esters were used for the entire study of the physical constants.

DETERMINATION OF VAPOR PRESSURE

The vapor pressure curve for each methyl ester was determined by the dynamic method described by Ramsay and Young (*1*); which was found to give very accurate results for all esters, including those which are normally solid at room temperature. This method was chosen because it requires a very small amount of material (approximately 1 ml.) which can be recovered, providing no decomposition takes place during the determination.

In Figures 1 and 2 are shown the vapor pressure curves for the pure methyl esters of the more commonly occurring fatty acids. The straight-line curves, drawn on semilog paper, were obtained by plotting the reciprocal of the absolute temperature × 1000 against the log of the pressure in millimeters of mercury. The curves, thus constructed, were extrapolated to 760-mm. pressure,

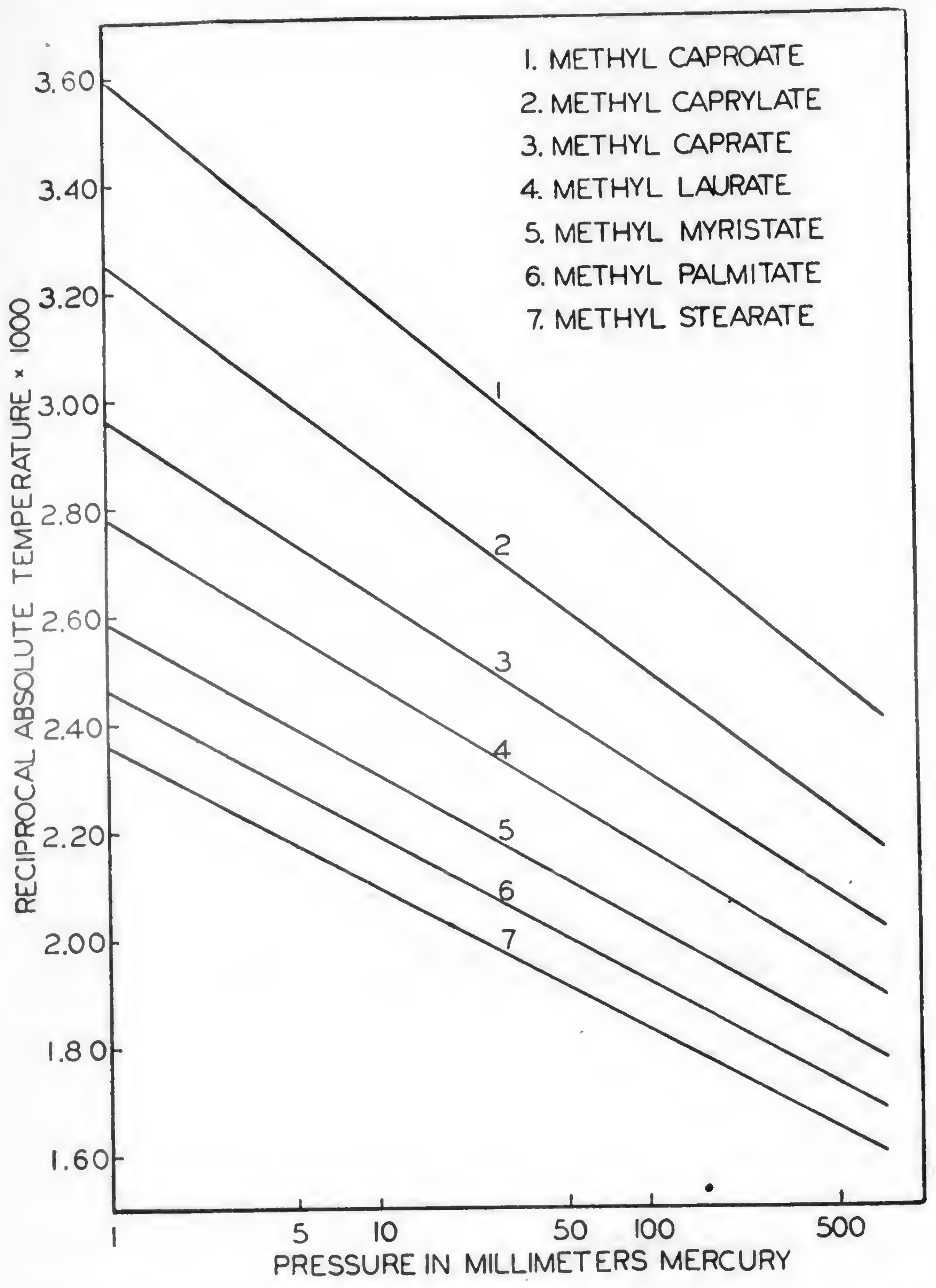


Figure 1. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

since in the majority of the cases decomposition occurs far below that pressure. Table I gives the approximate decomposition pressures and temperatures for each of the methyl esters studied. Table II shows the boiling points for each of the esters at several different pressures.

CONCLUSIONS

From the data collected, several significant conclusions may be drawn.

With the exception of the C_{18} series, whose vapor pressure curves as shown by Figure 2 are practically identical, it is possible to identify a pure ester fraction by means of its vapor pressure curve. With the above information available, it is an easy matter to find the boiling point of an unknown ester at several different pressures, and then by superimposing the curve thus constructed on one of the above standard curves, to identify the ester. Such identifications can be accomplished in a very short time and with a considerable degree of accuracy. The purity of a known ester may be determined in the same way. The ester is pure if the constructed curve can be superimposed on the standard vapor pressure curve of the ester in question. Any slight deviation from purity will be recognized immediately, inasmuch as the two curves will not coincide.

Table I. Decomposition Pressures and Temperatures of Methyl Esters of Fatty Acids

Ester	Pressure, Mm. Hg	Temperature, ° C.
Methyl caproate	>760	>150
Methyl caprylate	>760	>193
Methyl caprate	>760	>224
Methyl laurate	160	204
Methyl myristate	60	205
Methyl palmitate	25	151
Methyl stearate	18	221
Methyl oleate	16	217
Methyl linolate	11	208

Table II. Boiling Points of Methyl Esters at Various Pressures

Ester	Pressure in Millimeters of Hg						
	2	4	6	8	10	20	40
	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Methyl caproate	15	26	33	38	42	55	70
Methyl caprylate	45	58	65	71	76	89	106
Methyl caprate	77	89	97	103	108	123	139
Methyl laurate	100	113	121	128	134	149	166
Methyl myristate	127	141	150	157	162	177	197
Methyl palmitate	148	162	172	177	184	202	[a]
Methyl stearate	166	181	191	199	204	[a]	[a]
Methyl oleate	166.2	182	192	199.5	205.3	[a]	[a]
Methyl linolate	166.5	182.4	193	199.9	206	[a]	[a]

[a] Decomposes.

It is possible to adjust the pressure and hence the boiling temperature of a fractional distillation to optimum or nearly optimum conditions by making use of the recorded decomposition pressures and temperatures. The authors believe that during a fractional distillation there is a tendency toward unnecessary superheating of the material to be distilled. This tendency can be eliminated through the use of decomposition data on the various esters.

LITERATURE CITED

(1) Ramsay and Young, *J. Chem. Soc.*, 47, 42 (1885).
(2) Whitmore, F. C., and Lux, A. P., *J. Am. Chem. Soc.*, 54, 3448 (1932).

Journal Series Paper No. 1241, Pennsylvania Agricultural Experiment Station.

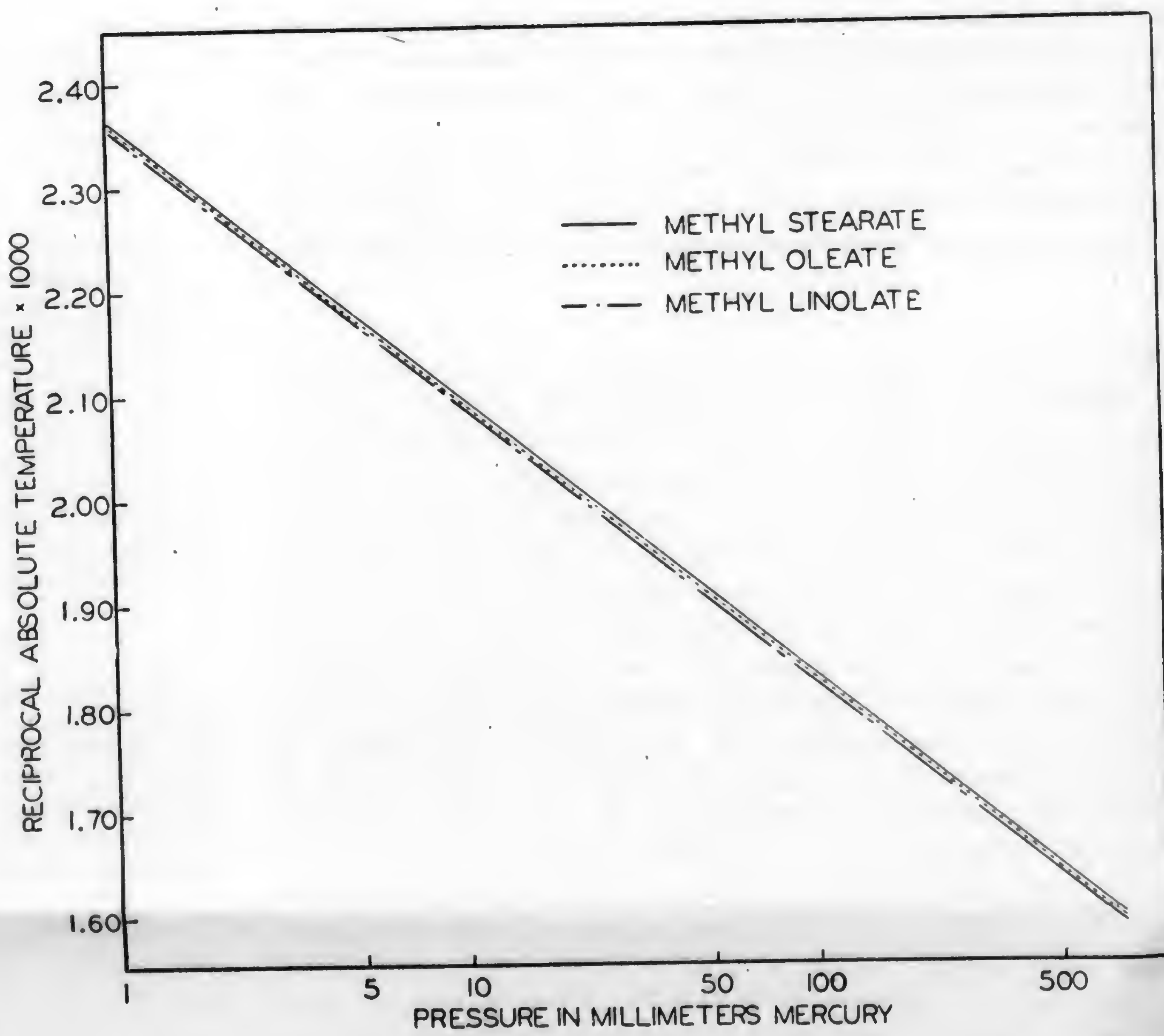


Figure 2. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

---

Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity as Fumigants[1]

H. L. King and D. E. H. Frear,[2] *Agricultural Experiment Station, Pennsylvania State College, State College, Pa.*

The possibility of using certain pyridine derivatives as fumigants was suggested to the authors by the work of Jewson & Tattersfield (1922), who used pyridine as a fumigant for certain mites in fungus cultures. With this work in mind, pyridine and 12 of its derivatives were tested as fumigants against the common red spider, *Tetranychus telarius* (L.). These preliminary tests indicated definite relationships between chemical structure and toxicity. A more extensive experiment using the confused flour beetle, *Tribolium confusum* Duv., as a test insect was then planned. During the time required for the development of a culture of flour beetles, 28 compounds were tested as fumigants against the large milkweed bug, *Oncopeltus fasciatus* (Dall.), quantities of which were readily available. These tests were discontinued as soon as the flour beetles were ready to use. The results reported in this paper include the preliminary tests against red-spiders and milkweed bugs as well as the final tests against flour beetles.

The pyridine derivatives tested were furnished by the Reilly Tar and Chemical Corporation and have been discussed in detail in an earlier paper (King & Frear, 1943). All the materials tested are listed in table 2.

Procedures.—*Greenhouse red spiders*—Two 6-inch provision jars were used for each fumigation chamber, one jar being inverted over the other and the two sealed together with cellulose tape. The total volume of such a chamber was 4800 ml. Potted string bean plants infested with mites were placed in the chambers, the required amount of fumigant introduced and the chamber sealed for five hours. At the end of this interval the chambers were opened and the plants were exposed to fresh air for 24 hours before making counts. After some preliminary trials a concentration of 3.0 milligrams of toxicant per liter of space was chosen. The temperature was maintained at 25° C. and duplicate tests were made of all materials. The average infestation was 85 mites per plant.

Milkweed bugs.—Four-liter erlenmeyer flasks (actual capacity 4500 ml.) with paraffined rubber stoppers were used as fumigation chambers. The required amount of fumigant, measured with a pipette and absorbed on a small piece of cellucotton was dropped into the flask. The flask was stoppered for an hour to allow the fumigant to volatilize. Twenty milkweed bugs (10 of each sex) were then introduced into the chamber and exposed to the fumigant for five hours. During the series of fumigations the temperature ranged from 27° to 30° C. After exposure the bugs were removed to a clean recovery cage containing fresh food and water. Mortality records were taken after 48 hours. Since the numerous controls showed no mortality the per cent kill in the test groups was reported directly as such.

Each material was tested first at a concentration of 2.0 milligrams per liter. Materials giving a high kill at this concentration were retested at lower concentrations, while the less effective compounds were retested at higher levels. Each compound was tested only once at any one concentration. For 24 of the materials. the results were such that they gave reasonably straight lines when plotted on logarithmic-probability paper. Median lethal concentrations were estimated for these compounds.

Flour beetles.—The technique was similar to that described above for the fumigation of milkweed bugs. The flour beetles were confined in small cylindrical cages of fine wire screen. During the five-hour exposure period these cages were suspended from the rubber stoppers of the chambers. The beetles were placed in clean whole wheat flour for the recovery period and mortality counts were made one week after exposure. Fifty beetles, selected at random, were used for each test. Each material was tested at a concentration of 5.0 milligrams per liter and then at higher or lower levels, as indicated by the results of the first test. An attempt was made to cover the range of toxicity for each material. In many cases several replications were made at critical concentrations. The number of concentrations at which each material was tested and the number of beetles counted are listed in table 2. Of the 42 materials tested, median lethal concentrations were estimated for 30. At the temperatures which obtained during these tests, *i.e.* 24°–30° C., the remaining 12 materials were not toxic, at the saturation point.

---

[1] Authorized for publication on April 3, 1944 as Paper No. 1228 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Former Reilly Research Fellow and Professor of Agricultural and Biological Chemistry, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation, of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry in cooperation with the Department of Zoology and Entomology, and at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

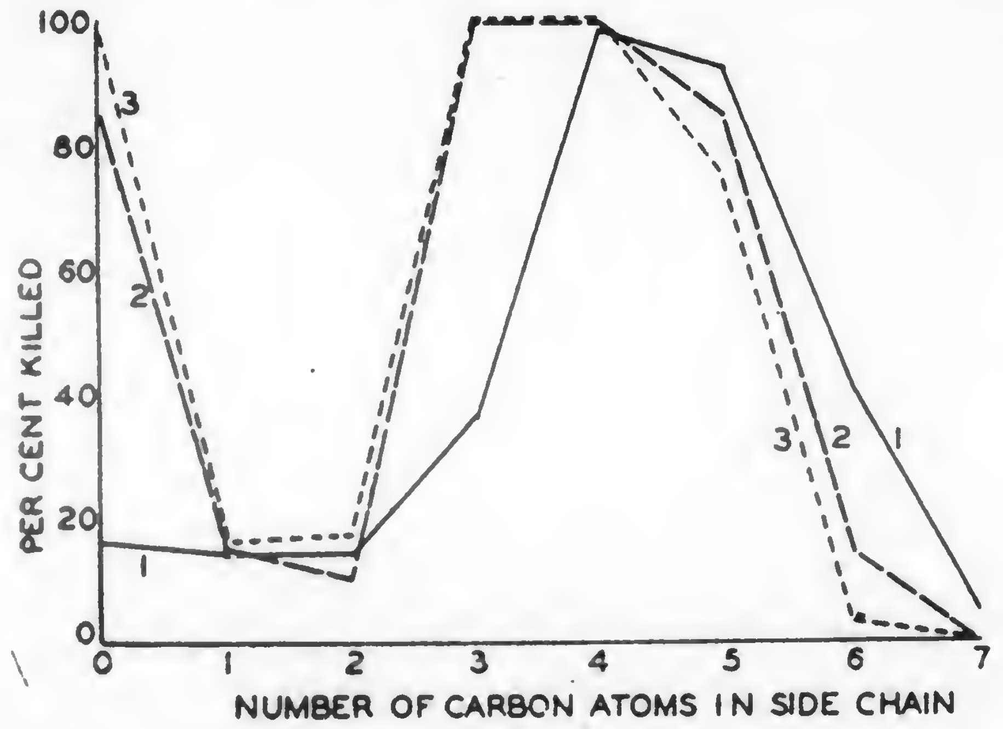


FIG. 1. (1) Effect of length of side chain on toxicity of 2-n-alkyl-pyridine fumigants to greenhouse red spider. Concentration 3.0 mg./l. (2)—Effect of length of side chain on toxicity of 2-n-alkyl-pyridine fumigants to milkweed bugs. Concentration 2.0 mg./l. (3)—Effect of length of side chain on toxicity of 2-*n*-alkyl-pyridine fumigants to flour beetles. Concentration 10 mg./l.

DISCUSSION OF RESULTS.—In this as well as in earlier papers (1944, 1944a) emphasis was placed on a study of the relationships between length and position of the side chains and toxicity to various organisms. Figure 1 (1) illustrates the effect of the length of the side chain on the toxicity of 2-*n*-alkyl-pyridine fumigants to red spider and shows the peak of toxicity that occurs at the butyl and amyl derivatives. At the time these tests were made the complete series of 4-alkyl-pyridines was not available. 4-*n*-amyl-pyridine and 4-*n*-propyl-pyridine were available, however, and tests of these two compounds indicated that they were more toxic than the corresponding 2-alkyl derivatives. Tests of a partial series of 2-*n*-alkyl—6-methyl-pyridines indicated that the toxicity of this series was less than that of either the 2- or 4-monosubstituted series.

Figure 1 (2) illustrates the effect of length of side chain on the toxicity of 2-*n*-alkyl-pyridine fumigants to milkweed bugs. It will be observed that a peak of toxicity occurs at the propyl and butyl derivatives and that pyridine, the first member of the series, is also relatively toxic. The relative toxicity of pyridine in this experiment was much greater than in the tests using red spiders, as will be noted by comparison of curves 1 and 2.

Median lethal concentrations were established for 27 of the materials tested against milkweed bugs. Compounds which were found to have a median lethal concentration less than 0.5 mg./l. were, in order of decreasing toxicity: 4-*n*-amyl-piperidine, 2-*n*-hexyl-piperidine, quinoline and 4-*n*-amyl-pyridine. Compounds with median lethal concentrations from 0.5 to 1.9 mg./l. were 4-*n*-butyl-pyridine, 2-*n*-butyl-pyridine, 4-*n*-propyl-pyridine, 2-*n*-propyl-pyridine, 4-isopropyl-pyridine, 4-ethyl-pyridine, pyridine, 2-*n*-amyl-pyridine and 2-butyl-6-methyl-pyridine. Compounds with median lethal concentrations between 2.0 mg. and 3.0 mg./l were 2-amyl, 6-methyl-pyridine, 4-mixed-hexyl-pyridines; 2,5-dimethyl-pyridine and 2,4-dimethyl-pyridine. Compounds with median lethal concentrations ranging from 3.0 mg./l to 4.0 mg./l were 2-isopropyl-pyridine; 2-*n*-hexyl-pyridine; 2-ethyl-6-methyl-pyridine; 3-methyl-pyridine; 2-ethyl-pyridine; 2,6-dimethyl-pyridine; 2-mixed-hexyl-pyridines, 2-hexyl-6-methyl pyridine and 2-*n*-heptyl-pyridine. Compounds with median lethal concentrations between 4.0 and 10.0 mg./l. were β,β′-dichloroethyl ether; carbon disulfide; methyl acetate; 2-ethanol-pyridine and 2-*n*-octyl-pyridine. Compounds with median lethal concentrations above 10.0 mg./l were 2-methyl-pyridine; 2-(3-octyl)-pyridine; 2-(2-me-octyl)-pyridine; 2-(6-undecyl)-pyridine; 2-mixed-undecyl-pyridines; 4-*n*-hexyl-pyridine; ethylene dichloride and carbon tetrachloride.

In these tests it was again observed that the 4-substituted alkyl pyridines were more toxic than the 2-substituted compounds and that the disubstituted compounds were less toxic than the corresponding monosubstituted compounds. It was also observed that the two piperidines tested were more toxic than the corresponding pyridines.

Table 1.—Concentrations of fumigants required to kill 50 per cent and 90 per cent of flour beetles.

COMPOUND	CONCENTRATION (IN MG./L) REQUIRED TO KILL	
	50%	*90%*
Pyridine	7.15	8.35
2 methyl-pyridine	10.14	13.00
2 ethyl-pyridine	11.32	14.26
2-*n*-propyl-pyridine	4.81	5.83
2-*n*-butyl-pyridine	6.40	8.73
2-*n*-amyl-pyridine	10.81	1836.[1]
2-*n*-hexyl-pyridine	966.0[1]	15310.[1]

[1] Values above saturation point.

Figure 1 (3) shows the effect of the length of the side chain on the toxicity of the 2-*n*-alkyl-pyridine fumigants to flour beetles. As in the milkweed bug tests, a peak of toxicity occurred at the propyl and butyl derivatives and pyridine itself was relatively toxic.

For a more critical study, the data obtained in the tests of the 2-*n*-alkyl-pyridines against flour beetles were plotted and analyzed according to the method of Bliss (1935). The equation of the regression line for each compound was computed and from the regression equation the concentrations required to kill 50 per cent and 90 per cent of the beetles were calculated. These concentrations are listed in table 1, from which it may be seen that 2-*n*-propyl-pyridine was the most toxic member of the series.

The final results of the complete series of flour beetle tests are given in table 2. Median lethal concentrations were estimated for all compounds tested, except the alkyl derivatives with 6 or more carbon atoms in the side chains. These compounds were not toxic at the saturation point. Four compounds: 2-*n*-amyl-pyridine; 4-*n*-butyl-pyridine, 4-*n*-amyl-pyridine and 4-*n*-amyl-piperidine, gave erratic results which were interpreted as indicating some degree of protective stupefaction.

From the values obtained for median lethal concentrations in the series of 4-*n*-alkyl-pyridines, it is seen that the peak of toxicity in this series occurred at the propyl derivative, as in the 2-*n*-alkyl-series. It is also seen that the members of the 4-substituted series were more toxic than the corresponding 2-substituted isomers. In the incomplete series of 2-*n*-alkyl,6-methyl-pyridines, the butyl compound was the most toxic, but since the propyl derivative was not available, the exact location of the peak was not determined.

Table 2.—Effects of some N-heterocyclic compounds as fumigants against the confused flour beetle.

COMPOUND TESTED	CONCENTRATIONS TESTED	BEETLES COUNTED	MEDIAN LETHAL CONCENTRATION MG/L
Pyridine	8	800	7.2
2-methyl-pyridine	8	1000	10.1
2-ethyl-pyridine	11	800	11.3
2-*n*-propyl-pyridine	10	800	4.8
2-isopropyl-pyridine	5	400	14.5
2-*n*-butyl-pyridine	7	800	6.4
2-*n*-amyl-pyridine	9	1200	10.8[2]
2-*n*-hexyl-pyridine	5	500	—[1]
2-mixed-hexyl-pyridine	6	450	—[1]
2-*n*-heptyl-pyridine	5	350	—[1]
2-*n*-octyl-pyridine	5	350	—[1]
2-(3-octyl)-pyridine	5	350	—[1]
2-(2-me-octyl)-pyridine	5	300	—[1]
2-(6-undecyl)-pyridine	5	350	—[1]
2-mixed-undecyl-pyridine	5	300	—[1]
3-methyl-pyridine	5	650	5.4
4-ethyl-pyridine	6	1200	7.2
4-*n*-propyl-pyridine	10	1150	3.5
4-isopropyl-pyridine	9	850	3.8
4-*n*-butyl-pyridine	10	1200	4.4[2]
4-*n*-amyl-pyridine	14	1200	8.0[2]
4-(3-pentyl)-pyridine	4	350	29.0
4-*n*-hexyl-pyridine	6	350	—[1]
4-mixed-hexyl-pyridine	5	350	—[1]
2-methyl-4-methyl-pyridine	7	750	9.0
2-methyl-5-methyl-pyridine	6	600	6.6
2-methyl-6-methyl-pyridine	10	750	6.5
2-ethyl-6-methyl-pyridine	9	1100	9.0
2-butyl-6-methyl-pyridine	6	450	4.5
2-amyl-6-methyl-pyridine	6	350	—[1]
2-hexyl-6-methyl-pyridine	6	350	—[1]
2-ethanol-pyridine	6	750	35.0
2-*n*-hexyl-piperidine	11	1000	11.0
4-*n*-amyl-piperidine	10	1500	7.6[2]
Quinoline	5	450	60.0
Carbon disulfide	5	250	55.0
Methyl acetate	5	250	82.0
Ethyl acetate	5	250	90.0
β,β′-Dichloroethyl ether	4	250	1.3
Ethylene dichloride	4	250	19.0
Carbon tetrachloride	8	400	66.0

[1] Not toxic at saturation point.
[2] Erratic results. See text.

The effect of hydrogenation was again illustrated by comparisons of the two piperidines with the corresponding pyridines. The median lethal concentration of 2-*n*-hexyl-piperidine was found to be 11.0 milligrams per liter, but 2-*n*-hexyl-pyridine was not toxic at the saturation point. A similar effect was not observed in a comparison of 4-*n*-amyl-piperidine and 4-*n*-amyl-pyridine.

The group of non-heterocyclic compounds listed at the end of table 2 was included for two reasons. First, it was desired to compare the alkyl pyridines with some standard fumigants such as carbon disulfide, ethylene dichloride and dichloroethyl ether. A comparison of the median lethal concentrations listed in table 2 for these compounds with those obtained for the *N*-heterocyclic compounds shows that dichloroethyl ether was more effective than any of the *N*-heterocyclic compounds but that none of the other standard fumigants tested was as toxic as the better heterocylcic compounds.

Second, it was desired to determine median lethal concentrations for a few compounds which had been tested by other workers, as a check on the resistance of the flour beetle culture used and on the technique employed. The compounds chosen were selected from a list of materials discussed by Shepard *et al.* (1937) in reporting an experiment in which the technique was similar to that used in the work reported here. The above authors obtained as median lethal concentrations the following values: carbon disulfide, 61 mg./l.; methyl acetate, 82 mg./l.; ethyl acetate 83 mg./l; β,β'-dichloroethyl ether 1.8 mg./l.; ethylene dichloride, 37.5 mg./l.; carbon tetrachloride 185 mg./l. comparison of these values with those given in table 2 shows that the results obtained in the two experiments for carbon disulfide, methyl acetate and ethyl acetate agree closely. The difference in results obtained for ethylene dichloride was probably caused by the difference in the time between exposure and counting. Richardson & Casanges (1942) report that the effect of ethylene dichloride on flour beetles increases during the post-fumigation period to the extent that 40 to 60 per cent of the beetles are sometimes killed between the tenth and twentieth days after exposure. In the light of this work it would be expected that the mortality counts made 40 hours after exposure by Shepard and his associates would indicate a higher median lethal concentration for this compound than the counts made one week after exposure in the work reported here.

A further check on the accuracy of the results reported in this paper is afforded by the work of Richardson & Haas (1932). These authors, using an entirely different technique, found the median lethal concentration of pyridine to *Tribolium* to be approximately 7.5 milligram per liter when the exposure period was 5 hours. This is within 0.35 mg. of the value reported in the present paper.

In table 3 a summarized comparison of the 2-alkyl-pyridines and 4-alkyl-pyridines is presented. It will be noted that of the 14 pairs of data listed, the 4-substituted compound is the more toxic in eleven cases, and in the other three cases the difference is insignificant.

Table 3.—Effects of point of attachment of alkyl side chain on the toxicity of alkyl pyridine fumigants to milkweed bugs and flour beetles.

LENGTH OF CHAIN	CONCENTRATION USED	PER CENT KILL BY	
		2-substituted compound	4-substituted compound
		Milkweed bug	
Ethyl	2 mg./l	10	100
n-propyl	2	100	100
is propyl	2	0	100
n-butyl	1	65	100
n-amyl	2	85	100
n-hexyl	2	15	40
mixed hexyl	2	0	45
		Flour beetle	
Ethyl	5	2	6
n-propyl	5	65	83
isopropyl	5	1	37
n-butyl	5	26	74
n-amyl	5	2	23
n-hexyl	5	2	0
mixed hexyl	5	6	0

Table 4 is a similar summary of paired data showing comparisons between compounds having straight side chains and those having branched side chains. Among those compounds with short side chains there is a tendency for the normal compounds to be considerably more toxic than the branched isomer. As the length of the chain increases, however, the difference between straight and branched chains decreases. In the hexyl and octyl compounds the effect seems to be reversed, the branched chain compounds being more toxic than the normal isomers.

A comparison of curves 1, 2 and 3 shows a striking similarity in the action of the 2-*n*-alkyl-pyridine fumigants on the three organisms studied, with the general range of the peak of toxicity at the compounds having 3 or 4 carbon atoms in the side chain. From a practical point of view, it is interesting to note that for fumigation of milkweed bugs and flour beetles there is little to be gained by the addition of the side chain to the pyridine base, since pyridine itself was almost as toxic as any of the alkyl derivatives. Against red spiders, however, pyridine was relatively low in toxicity. It was expected that mites would react to fumigants in a manner different from insects, so the difference in response to pyridine was not surprising. The similarity of the curves for red spiders and for the two insects was entirely unexpected, however. It may be an indication that the alkyl pyridines tested are capable to some extent of penetrating directly through the cuticle, as Glover & Richardson (1936) have demonstrated with nicotine, pyridine and piperidine.

Table 4.—Comparison of toxicities of alkyl pyridine fumigants having straight and branched side chains.

POSITION AND LENGTH OF SIDE CHAIN	TEST ORGANISM	CONCENTRATION USED	PER CENT KILL BY	
			Normal chain compound	Branched chain compound
2-propyl	Red spider	3 mg./l.	35	5
2-propyl	Milkweed bug	2	100	0
2-propyl	Flour beetle	5	65	1
4-propyl	Milkweed bug	0.5	25	5
4-propyl	Milkweed bug	1	35	10
4-propyl	Flour beetle	2	16	0
4-propyl	Flour beetle	5	83	37
4-amyl	Flour beetle	10	52	12
2-hexyl	Red spider	3	41	30
2-hexyl	Milkweed bug	2	15	0
2-hexyl	Milkweed bug	3	20	10
2-hexyl	Flour beetle	5	2	6
2-hexyl	Flour beetle	40	12	14
4-hexyl	Milkweed bug	2	40	45
4-hexyl	Milkweed bug	3	0	75
4-hexyl	Flour beetle	10	0	17
2-octyl	Milkweed bug	5	35	70
2-octyl	Milkweed bug	10	55	35
2-octyl	Flour beetle	20	1	1

SUMMARY.—The work reported in this paper includes tests of 13 pyridine derivatives as fumigants against greenhouse red spiders, tests of 28 pyridine derivatives as fumigants against the large milkweed bug and tests of 34 materials related to pyridine as fumigants against the confused flour beetles. Emphasis was placed on a study of the relationship between toxicity and length, position and type of side chain. In all of the tests carried out it was evident that in a series of 2-*n*-alkyl-pyridines a pronounced peak of toxicity occurred at the propyl- or butyl-derivative. Alkyl pyridines with side chains in the 4-position were more toxic than the 2-substituted isomers and compounds with normal side chains were usually more toxic than the branched isomers. The two alkyl piperidines available were more toxic than the corresponding pyridines. The relative toxicities of the materials tested against red spiders showed a high degree of correlation with the action of the same compounds against the two species of insects studied.—4-6-44.

LITERATURE CITED

Bliss, C. I. 1935. The calculation of the dosage-mortality curve. Ann. Appl. Biol. **22**(1): 134–67.

Glover, L. H., and C. H. Richardson. 1936. The penetration of gaseous pyridine, piperidine and nicotine into the body of the American cockroach, *Periplaneta americana*, L. Iowa State Coll. J. Sci. **10**(3): 249–60.

Jewson, S. T., and F. Tattersfield. 1922. The infestation of fungous cultures by mites. Ann. Appl. Biol. **9**(3–4): 213–40.

King, H. L., and D. E. H. Frear. 1943. Relation of chemical constitution of some N-heterocyclic compounds to toxicity to *Tetranychus telarius* (L.). JOUR. ECON. ENT. **36**(2): 263–5.

King, H. L., and D. E. H. Frear. 1944. Relation of chemical constitution of some N-heterocyclic compounds to toxicity against *Tribolium confusum* Duval. JOUR. ECON. ENT. **37**(5): 634–7.

King, H. L., D. E. H. Frear and L. E. Dills. 1944. Relation of chemical constitution of some N-heterocyclic compounds to toxicity to *Aphis rumicis* L. JOUR. ECON. ENT. **37**(5): 637–40.

Richardson, H. H., and A. H. Casanges. 1942. Toxicity of acrylonitrile, chloroacetonitrile, ethylene dichloride and other fumigants to the confused flour beetle. JOUR. ECON. ENT. 34(5): 664–8.

Richardson, C. H., and L. E. Haas. 1932. The relative toxicity of pyridine and nicotine in the gaseous condition to *Tribolium confusum* Duval. Iowa State Coll. Jour. Sci. **6**(3): 287–98.

Shepard, H. H., D. L. Lindgren, and E. L. Thomas. 1937. The relative toxicity of insect fumigants. Minn. Agr. Expt. Sta. Tech. Bul. **120**.

Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity Against *Tribolium confusum*[1]

H. L. KING and D. E. H. FREAR,[2] *Agricultural Experiment Station, Pennsylvania State College State College, Pa.*

During the course of a comprehensive investigation of the relationship between chemical structure and toxicity in a series of pyridine derivatives,[3] it was desired to learn something of the nature of the toxic action of these compounds against the confused flour beetle, *Tribolium confusum* Duval, and on the basis of this work to establish some relationship between chemical structure and toxicity to this insect.

Although many N-heterocyclic compounds have been tested as insecticides by various workers, among whom may be mentioned Tattersfield & Gimingham (1927), Richardson and his associates (1930, 1933, 1936), and King & Frear (1943), little is known regarding the toxicity of these materials as stomach poisons, emphasis having been placed on contact action in most cases.

The use of the confused flour beetle, *Tribolium confusum* Duval, as a test organism for toxicity experiments, although never widely practiced, is not a new method, these insects having been used by Dobroscky (1935) in measuring the relative toxicity of lead arsenate and natural cryolite and by Fassig & Campbell (1937) in studying the effectiveness of a series of homologs of paris green. A modification of the methods used by these workers was found to be satisfactory for this experiment. Although in any experiment in which the test insect is intimately in contact with the toxicant there is the possibility of action as fumigant, contact and stomach poisons, the fumigant action was reduced to a minimum in this experiment by the use of non-volatile salts.

MATERIALS USED.—The free N-heterocyclic bases: 2-ethyl-pyridine, 4-*n*-propyl-pyridine, 4-*n*-amyl-pyridine, 2-*n*-hexyl-pyridine, 2-mixed-undecyl-pyridine, 2,6-lutidine and quinoline were furnished by the Reilly Tar and Chemical Corporation. While the commercial method of preparation of the samples used is not available for publication, a suitable laboratory synthesis of the short-chain alkyl-pyridines has been published by Tchitchibabine (1936), and Knight & Shaw (1938) have shown that higher members of the series can be prepared by a modification of Tchitchibabine's method.

Since flour beetles live and reproduce normally in a diet of whole wheat flour, it was necessary to incorporate the experimental compounds into the flour in such a way that fumigant action could be prevented, without interfering with the action of material upon the insects. With this in mind it was decided to use salts of the bases, rather than the free bases, in this experiment. The ability of pyridine to form salts with mineral acids has been known practically as long as pyridine itself, the formation of pyridine sulfate having been mentioned by Anderson (1858), the discoverer of pyridine. Some confusion regarding the exact composition of this salt has existed in the literature, however, until very recently, when the work of Peakin (1940) indicated that in the ternary system $C_5H_5N-H_2SO_4-H_2O$, the only solid sulfate capable of isolation at 20° C., was the acid salt $C_5H_5N\cdot H_2SO_4$, and that a solution of pyridine, sulfuric acid and water in any proportions yields on evaporation $C_5H_5N\cdot H_2SO_4$ or a mixture of this salt and sulfuric acid. Assuming that the alkyl pyridines and quinoline would react similarly to pyridine, a series of sulfates was prepared by adding concentrated sulfuric acid to an excess of the base and cooling the reaction mixture. The sulfates of quinoline, 4-*n*-propyl-pyridine, 4-*n*-amyl-pyridine and 2,6-lutidine formed crystalline precipitates, which were recrystallized from alcohol and washed with acetone. The sulfates of 2-ethyl-pyridine, 2-*n*-hexyl-pyridine and 2-mixed-undecyl-pyridine did not form precipitates but separated as viscous, water-soluble liquids.

In addition to its ability to form salts with mineral acids, pyridine also forms complex compounds with metallic salts. The formation of many such double salts has been discussed in detail by Lang (1888) and Pincussohn (1897). Following the general methods of these workers, and again assuming that the alkyl pyridines and quinoline would react similarly to pyridine itself, a series of copper chloride double salts was prepared by adding 50 grams of the free base to the calculated amount of copper chloride, dissolved in 200 ml. of alcohol. Crystalline precipitates formed in each case. These crystals were of the general type $(RC_5H_4N)_2\cdot CuCl_2$, containing two molecules of the pyridine derivative to one of the metallic chloride, since the calculated amount of $CuCl_2$ reacted completely with the pyridine derivatives.

Table 1.—Number of days required to kill 50 per cent of adult flour beetles. (Ration Containing 10 Per Cent of Toxicant)

TOXICANT	DAYS
2 ethyl-pyridine sulfate	30
4-*n*-propyl-pyridine sulfate	55
4-*n*-amyl-pyridine sulfate	53
2-*n*-hexyl-pyridine sulfate	70
2 mixed undecyl-pyridine sulfate	100
quinoline sulfate	80
nicotine sulfate	66
check (no appreciable mortality after 100 days)	

In addition to the sulfate and the copper chloride double salt mentioned above, three crystalline salts—a picrate, a salicylate, and a zinc chloride double salt—were made of 4-*n*-propyl-pyridine. The zinc chloride salt was prepared by adding the free base to the calculated amount of zinc chloride, dissolved in water, and was apparently of the same type as the copper chloride salt. In the preparation of the picrate and the salicylate, however, it was assumed that the base, like the parent compound, would react with one molecule of the acid (Allen, 1912). The picrate was prepared by adding the base to an aqueous solution of the acid, and the salicylate by adding the base to an alcoholic solution of the acid.

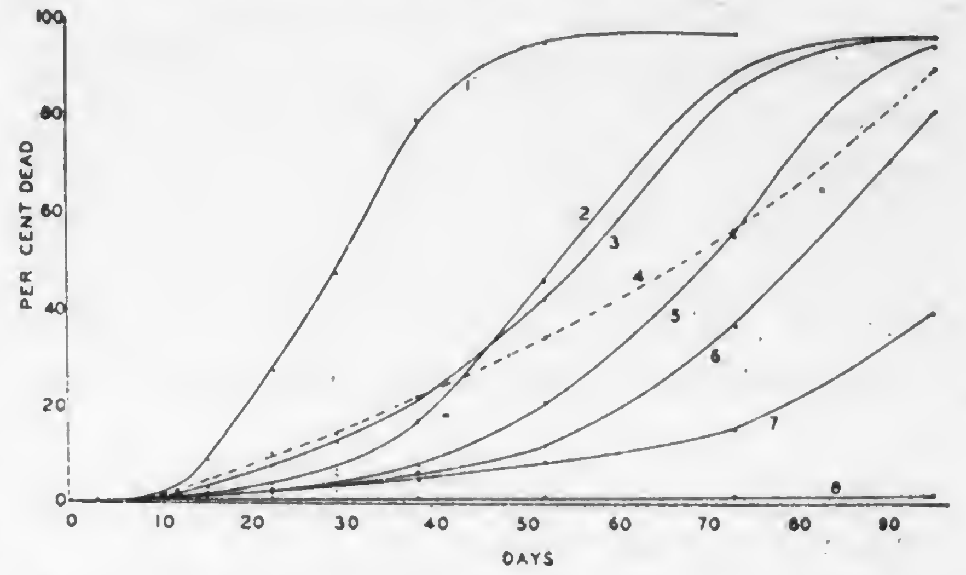


FIG. 1.—Effect of alkyl pyridine sulfates and related materials as stomach poisons against *Tribolium confusum*. Whole wheat flour 90%; test materials 10%.

1. 2-ethyl-pyridine sulfate
2. 4-*n*-amyl-pyridine sulfate
3. 4-*n*-propyl-pyridine sulfate
4. nicotine sulfate
5. 2-*n*-hexyl-pyridine sulfate
6. quinoline sulfate
7. 2-mixed-undecyl-pyridine sulfate
8. whole wheat flour controls

EXPERIMENTAL PROCEDURE AND RESULTS.—For each treatment, the required amount of crystalline toxicant was ground in a mortar and added to enough whole wheat flour to make 50 grams of ration. After mixing with a spatula, the ration was ground in a small Wiley mill to insure thorough mixing and crushing of all particles. The three test materials that were not crystalline were dissolved in alcohol and mixed with the flour while in solution. The solvent was evaporated off with frequent stirring of the mixture and the dried ration ground and mixed thoroughly. The mixture was divided into five 10-gram portions, each of which was placed in a 125 ml. beaker. One hundred adult flour beetles were added to each of these. The dead beetles were removed and counted, daily at first, but less frequently as the experiment progressed. The averages of the 5 replicates of each treatment, when plotted on coordinate paper, formed a smooth time-mortality curve, from which the time required to kill 50 per cent of the beetles was read without difficulty. This period of time was used as the standard of comparison for the various treatments considered.

The alkyl pyridine sulfates discussed above were prepared and tested during the period August 1 to October 30, 1942. At the start of the experiment the test beetles had been mature about two months, and were probably at the height of their resistance. The results of this test are given in table 1 and in figure 1.

---

[1] Authorized for publication on April 3, 1944, as Paper No. 1229 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Former Reilly Research Fellow and Professor of Agricultural and Biological Chemistry, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry in cooperation with the Department of Zoology and Entomology, and at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

[3] The results presented in this paper are taken from a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, The Pennsylvania State College, December 1942.

The remaining salts, i.e., the salicylate, picrate and metallic double salts, were prepared in August 1942, but were not tested against flour beetles until the period January 1–March 30, 1943 (Table 2). At this time the beetles which were available had been mature for six months and were not as resistant as those used in the earlier test, the decrease in vigor being quite apparent in a comparison of the check groups of the two tests. For this reason, direct comparisons between the two tests are not justifiable. Each test must be considered complete in itself and it must be remembered that the values obtained are relative values only, and are dependent upon the vigor of the insect culture at the time of the experiment.

Table 2.—Number of days required to kill fifty per cent of adult flour beetles.

CONCENTRATION OF TOXICANT	10%	20%	40%
4-*n*-propyl-pyridine sulfate		25	18
4-*n*-propyl-pyridine salicylate	11	10	10
4-*n*-propyl-pyridine picrate	9	9	9
4-*n*-propyl-pyridine zinc chloride	15	13	10
4-*n*-propyl-pyridine copper chloride	8	8	8
2 ethyl-pyridine copper chloride	9	9	8
4-*n*-propyl-pyridine copper chloride	8	8	8
4-*n*-amyl-pyridine copper chloride	9	9	9
2,6-lutidine copper chloride	9	8	6
quinoline copper chloride	10	9	8
starvation check	*8*		
Whole wheat flour alone	*22*		

DISCUSSION.—Although none of the sulfates listed in table 1 proved to be highly toxic against the confused flour beetle, it is obvious that marked differences existed within the series of compounds. The sulfate of 2-ethyl-pyridine, for example, was much more toxic than the other salts of this series. The sulfates of 4-*n*-amyl-pyridine and 4-*n*-propyl-pyridine were next in order of toxicity, being very nearly equal to each other. The sulfate of 2-*n*-hexyl-pyridine was much less toxic than the above salts and 2-mixed-undecyl-pyridine sulfate, the last of the series, was the least toxic compound studied. While it is impossible in an experiment of this kind to distinguish clearly between toxicity due to contact action and that produced by the ingested toxicant, the former is in general much more rapid than the latter. The curves obtained in this experiment indicate that the toxicity of the compounds tested was largely, if not entirely, due to stomach poison rather than contact action. The most important observation to be drawn from this experiment is that the order of toxicity of the series of compounds was just the reverse of the order obtained by the authors (1943) in contact spray experiments against the greenhouse red spider, probably indicating that the shorter chain compounds were more effective stomach poisons while the longer chain compounds were more effective contact sprays.

The results given in table 2 appear to indicate that many of the compounds were repellent to the beetles. The entire series of copper chloride double salts, for example, gave time-mortality curves not significantly different from each other or from the starvation check. It is significant, also, that the increase in concentration of toxicant from 10 per cent to 40 per cent did not have any appreciable effect on the mortality—another indication that a condition of more or less complete repellency obtained.

The results given by the various salts of 4-*n*-propyl-pyridine demonstrate to what an extent the inherent toxicity of the base may be modified by the rest of the molecule. The copper chloride double salt was completely repellent and the picrate, salicylate, zinc chloride double salt and sulfate gave results that may be taken as an indication of medium toxicity, medium repellency or both. With the available data, it is impossible to be sure of the exact nature of the action of these materials for which the time-mortality curves fall between those of the starved controls and those of the fed controls.

SUMMARY.—Seventeen salts of various pyridine derivatives were prepared and tested against adults of the confused flour beetle, *Tribolium confusum* Duval. A series of alkyl pyridine sulfates gave results indicating that the salts with short alkyl side chains were more toxic than those with long chains. The toxicity of these compounds appeared to be due largely to the material ingested, rather than to contact action. A series of alkyl pyridine copper chloride double salts appeared to be completely repellent to the beetles. Results obtained with three additional salts of 4-*n*-propyl-pyridine indicated that the picrate, salicylate and the zinc chloride double salt were slightly toxic or partially repellent, or both.

LITERATURE CITED

Allen's Commercial Organic Analysis, Fourth Edition, Vol. 6, 1912. Philadelphia, P. Blakiston's Son and Co., p. 134.

Anderson, Th. 1858. Ueber die Producte der trockenen Destillation thierischer Materien. Annalen der Chemie **105**(3): 355–9.

Craig, L. C., and C. H. Richardson. 1933. Insecticidal action in the nitrogen heterocyclic compounds. Iowa State Col. Jour. Sci. **7**(4): 477–86.

Dobroscky, Irene D. 1935. Preliminary report on the fluorine compounds as insecticides. JOUR. ECON. ENT. **28**(3): 627–37.

Fassig, W. W., and F. L. Campbell. 1937. Relative effectiveness of homologs of Paris Green against confused flour beetle. JOUR. ECON. ENT. **30**(4): 681–2.

Glover, L. H., and C. H. Richardson. 1936. The penetration of gaseous pyridine, piperidine and nicotine into the body of the American cockroach, *Periplaneta americana*, L. Iowa State Col. Jour. Sci. **10**(3): 249–60.

King, H. L., and D. E. H. Frear. 1943. Relation of chemical constitution of some N-heterocyclic compounds to toxicity to *Tetranychus telarius* (L.). JOUR. ECON. ENT. **36**(2): 263–65.

Knight, G. A., and B. D. Shaw. 1938. Long chain alkyl pyridines and their derivatives. New examples of liquid crystals. J. Chem. Soc. **1938**(Part I): 682–3.

Long, Wilhelm. 1888. Einwerkung von Pyridin auf Metallsalze. Ber. **21**(Part II): 1578–88.

Peakin, F. H. 1940. The sulfates of pyridine. J. Soc. Chem. Ind. Trans. **59**: 56–7.

Pincussohn, Ludwig. 1897. Über die Metallverbindungen des Pyridine und die Electrolyse des Pyridins. Zeitschrift für Anorganische chemie. **14**: 379–403.

Richardson, C. H., and H. H. Shepard. 1930. The insecticidal action of some derivatives of pyridine and pyrrolidine and of some aliphatic amines. J. Agr. Res. **40**: (11) 1007–15.

Tattersfield, F., and C. T. Gimingham. 1927. Studies on contact insecticides. V. The toxicity of the amines and N-heterocyclic compounds to *Aphis rumicis* L. Ann. Appl. Biol. **14**(2): 217–39.

Tchitchibabine, A. E. 1936. Tautomérie des homologues de la pyridine. II. Nouvelles synthèses dans la série de la pyridine. Bull. soc. chim., Sér. 5, **3**(2): 1607–32.

Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to *Aphis rumicis*[1]

H. L. KING, D. E. H. FREAR and L. E. DILLS,[2] *Agricultural Experiment Station*

A number of workers have studied the contact action of pyridine and picoline on aphids. The work of Tattersfield & Gimingham (1927), Richardson & Shepard (1930) and Richardson & Smith (1923) indicated that these materials were of little value as aphicides. The authors have found no tests of the higher members of the alkyl pyridine series reported in the literature. By courtesy of the Reilly Tar and Chemical Corporation, samples of a number of higher alkyl pyridines and other pyridine derivatives have been made available for testing. The data reported in this paper are the results of aphicidal tests of 37 of these materials (See Table 1). From this group of compounds, it was possible to separate three distinct homologous series with alkyl chains ranging from one to eight carbon atoms in length.

PREPARATION OF MATERIALS.—With the exception of 2-amino-pyridine, which was crystalline in nature, all of the materials were liquids at room temperature. The lower members of the series were readily soluble in water but the higher members were progressively less water-soluble and required some emulsifying agent to make suspensions suitable for testing. For uniformity *Orvus* wetting agent (a sodium lauryl sulfate paste—see Cupples 1940) was used in all spray suspensions at the rate of .025 gm. of paste per 100 ml. sample. This acted as a wetting agent in all samples and as an emulsifier where necessary.

PROCEDURE OF REARING AND TESTING.—A stock culture of bean aphids, *Aphis rumicis*, L., was maintained in the greenhouse on potted nasturtium plants. A few (in most cases three) days before testing, aphids were transferred from the stock culture to small nasturtium plants in pots. Each test pot contained two plants, with

[1] Authorized for publication on April 3, 1944 as Paper No. 1230 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Former Reilly Research Fellow, Professor of Agricultural and Biological Chemistry and Assistant Professor of Economic Entomology, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation, of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry and the Department of Zoology and Entomology, at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

an average over the entire experiment of 175 aphids per plant. The spraying was carried out in the spray chamber described by the senior authors (King & Frear 1943) in an earlier publication. For this experiment the chamber was moved into the laboratory, which was maintained at a more uniform temperature than the greenhouse, and the test plants, after being sprayed to the point of run-off, were placed in a constant temperature room maintained at 28±1° C. This procedure eliminated much of the variability experienced in preliminary tests conducted in the greenhouse. Counts were made as described by Hartzell & Wilcoxon (1932).

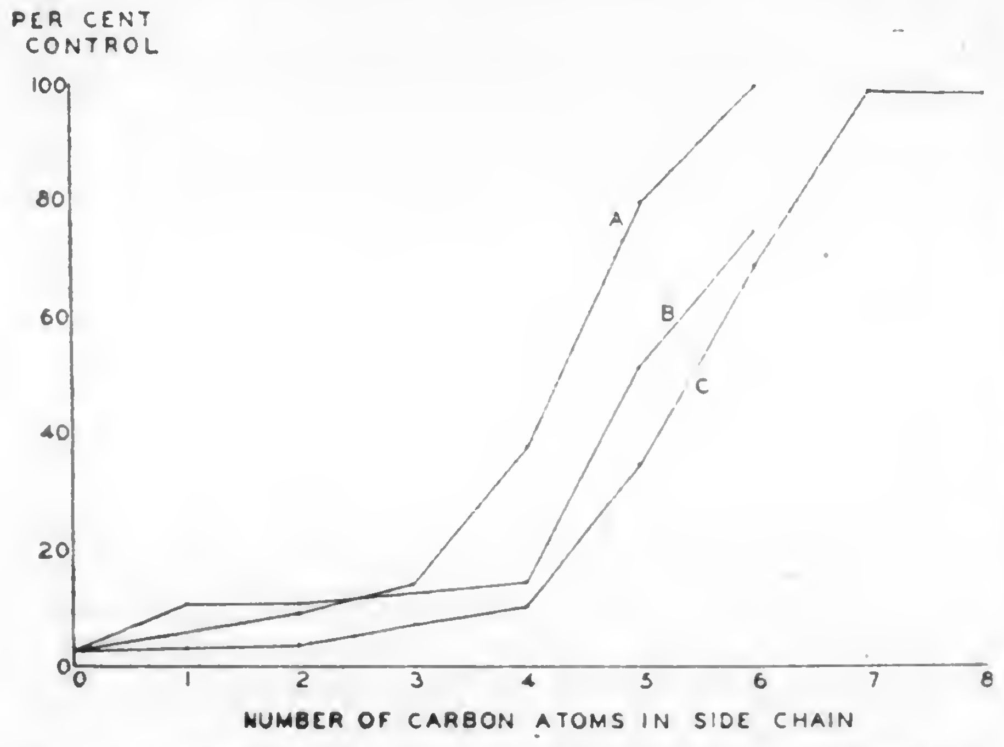


FIG. 1.—Effects of length and position of side chain on toxicity of alkyl pyridines to *Aphis rumicis*, L. Toxicant 1 per cent; *Orvus* wetting agent 0.025 per cent.

A. 4-*n*-alkyl-pyridines
B. 2-*n*-alkyl-6-methyl-pyridines
C. 2-*n*-alkyl-pyridines

EXPERIMENTAL DESIGN AND RESULTS.—Each material was tested at a concentration of 1.0 per cent, with 1.0 gm. of toxicant, 0.025 gm. of *Orvus* wetting agent paste and 99.0 ml. of distilled water per sample. Because of the large number of materials, it was impossible to complete a replicate on any given day. Approximately a third of the materials were tested each day, and the compounds were regrouped at the completion of each replicate to avoid an accumulation of errors caused by day-to-day variation in the aphid culture. With the exception of 2-amino-pyridine, of which only a very limited quantity was available, each material was tested on from 3 to 7 different days, depending on the degree of agreement obtained. As a control, a solution of *Orvus* in water was included in each day's run. With this as a reference, Abbott's per cent control (Abbott 1925) was calculated for each day's results. The averages obtained are tabulated.

DISCUSSION.—The most important results obtained in this experiment are summarized graphically in figure 1. In this figure, three homologous series of compounds are represented: a series of 2-*n*-alkyl-pyridines, a series of 4-*n*-alkyl-pyridines, and a series of 2-*n*-alkyl-6-methyl-pyridines. Comparison of the three curves shows at once that the 4-substituted alkyl pyridines were more toxic than the corresponding 2-substituted compounds.

Table 1.—Effects of sprays containing 1 per cent of various pyridine derivatives on *Aphis rumicis* L. and on nasturtiums.

MATERIAL	NO. OF DAYS TEST-ED	NO. OF APHIDS COUNT-ED	PER CENT CONTROL OF APHIDS	INJURY TO PLANTS
Pyridine	11	3438	2.4	none
2-methyl-pyridine	5	2193	2.9	none
2-ethyl-pyridine	5	2130	3.5	none
2-*n*-propyl-pyridine	3	1388	7.0	none
2-isopropyl-pyridine	5	1678	10.1	none
2-*n*-butyl-pyridine	3	1180	10.1	none
2-*n*-amyl-pyridine	4	1469	34.2	severe
2-*n*-hexyl-pyridine	4	1443	68.7	very severe
2-mixed-hexyl-pyridine	5	1728	53.3	severe
2-*n*-heptyl-pyridine	3	1055	98.2	very severe
2-*n*-octyl-pyridine	3	1032	97.4	very severe
2-(3-octyl)-pyridine	3	978	79.3	very severe
2-(2-me-octyl)-pyridine	3	799	97.5	very severe
2-(6-undecyl)-pyridine	4	1312	86.9	very severe
2-mixed-undecyl-pyridine	3	972	78.6	very severe
2-ethanol-pyridine	3	917	4.5	moderate
2-amino-pyridine	2	523	50.7	severe
3-methyl-pyridine	5	1891	8.4	none
4-ethyl-pyridine	3	1355	9.1	none
4-*n*-propyl-pyridine	4	1510	14.0	none
4-isopropyl-pyridine	4	1546	15.5	none
4-*n*-butyl-pyridine	4	1650	37.9	moderate
4-*n*-amyl-pyridine	5	2756	79.8	severe
4-(3-pentyl)-pyridine	3	1101	36.1	moderate
4-*n*-hexyl-pyridine	3	805	99.2	very severe
4-mixed-hexyl-pyridine	3	842	98.5	very severe
2-me-4-me-pyridine	4	1409	18.1	none
2-me-5-me-pyridine	5	1881	20.5	none
2-me-6-me-pyridine	7	2742	10.8	none
2-et-6-me-pyridine	7	2887	10.2	none
2-butyl-6-me-pyridine	5	1606	14.5	none
2-amyl-6-me-pyridine	7	3165	51.1	severe
2-hexyl-6-me-pyridine	4	1074	74.3	very severe
2-amino-6-me-pyridine	4	1255	58.5	severe
Quinoline	3	1057	76.4	very severe
2-*n*-hexyl-piperidine	3	1484	40.2	slight
4-*n*-amyl-piperidine	4	1101	81.7	severe

Likewise, they were more toxic than the 2, 6-, disubstituted derivatives. The general shape of the three curves indicates that regardless of the position of the substitution the toxicity of an alkyl pyridine increases markedly when the alkyl chain is increased to 5 or more carbon atoms. In the series tested this increase in toxicity was found to continue to the end of the series. The apparent peak shown in figure 1 for 2-*n*-heptyl-pyridine was proved to be within the limits of experimental error and replicated tests of 2-*n*-heptyl-pyridine and 2-*n*-octyl-pyridine at a concentration of 0.25 per cent indicated that at this lower concentration the octyl derivative was definitely more toxic than the heptyl compound, the two materials giving 84.4 and 65.9 per cent control, respectively.

The shape of the curves also indicates that the addition of a -CH_2- group to the side chain attached to the pyridine nucleus did not always produce the same increase in toxicity. At the lower end of the 2-*n*-alkyl-pyridine series, for example, the increase from one to two carbon atoms in the side chain (methyl to ethyl) increased the toxicity less than 1 per cent, while higher in the same series, the same increase (from amyl to hexyl) increased the toxicity 34 per cent. Similar relations may be observed in the other two series of compounds studied. The effect on toxicity of the methyl group in the 6 position in the 2-*n*-alkyl-6-methyl-derivatives was consistently low throughout the entire series.

It was observed in this series that toxicity was roughly parallel to insolubility in water, the more highly effective materials being those which formed emulsions with water.

A number of interesting points may be noted in connection with the miscellaneous materials which could not be grouped in any series. For example, the normal or straight-chain alkyl pyridines were generally more toxic than the corresponding branched-chain isomers. The propyl compounds were exceptions to this generalization, since the isopropyl derivatives were more toxic in each case than the normal isomers. Among the higher homologs, however, it will be seen that 2-*n*-hexyl-pyridine was more toxic than 2-mixed-hexyl-pyridine; 2-*n*-octyl-pyridine was more toxic than 2-(3-octyl)-pyridine; and 4-*n*-amyl-pyridine was more toxic than 4-(3-pentyl)-pyridine.

The amino derivatives, 2-amino-pyridine and 2-amino-6-methyl-pyridine are other interesting compounds which do not fit any series. The results given by these materials illustrate the marked increase in toxicity caused by the addition of an amino group to the pyridine nucleus, as compared to the slight effect of the addition of a methyl group. The high toxicity of the amino pyridines was noted by Wibaut (1934) and was reported by King & Frear (1943) in a study of the toxicity of these materials to the greenhouse red spider.

It was expected that 2-*n*-hexyl-piperidine and 4-*n*-amyl-piperidine would be more toxic than the corresponding pyridines, since Tattersfield & Gimingham (1927) as well as Richardson & Shepard (1930) found that hydrogenation of certain N-heterocyclic compounds increased toxicity markedly. In the present study, however, hydrogenation appeared to have no effect or to decrease toxicity, rather than to increase it.

A comparison of the three isomeric lutidines, or dimethyl pyridines, provides further proof that toxicity is enhanced more by alkyl substituents on the 3, 4 or 5 positions than on the 2 or 6 positions, since 2,4-dimethyl-pyridine and 2,5-dimethyl-pyridine gave twice as high control as 2,6-dimethyl-pyridine.

It is interesting to note that phytotoxic effect closely paralleled aphicidal action. The highest control obtained without injury to the plants was 20.5 per cent, given by 2,5-dimethyl-pyridine. 2-*n*-hexyl-piperidine gave 40.2 per cent control with only slight injury to the plants. All other compounds giving more than 20.5 per cent control of the insects caused burning of the foliage ranging from moderate to severe.

SUMMARY.—Pyridine and 36 related compounds were tested as contact insecticides against *Aphis rumicis*, L. In a series of 2-*n*-alkyl-pyridines it was found that compounds with 5 or more carbon atoms in the side chain were much more toxic than those with short chains, the toxicity increasing with the length of the substituent group. A series of 2-*n*-alkyl-6-methyl pyridines gave results closely parallel to those given by the 2-*n*-alkyl-pyridines. A series of 4-*n*-alkyl-pyridines was more toxic than the corresponding 2-*n*-alkyl-compounds. Normal or straight-chain alkyl compounds were generally more toxic than the branched chain isomers. The addition of an amino group to the pyridine nucleus increased the toxicity much more than the addition of a methyl group in the same position. Hydrogenation of two alkyl pyridines to the corresponding piperidines did not increase the toxicity. Phytotoxicity was generally parallel to aphicidal action.—4-6-44.

LITERATURE CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. JOUR. ECON. ENT. 18(2): 265–7.

Cupples, H. L. 1940. A list of commercially available detergents, wetting, dispersing, and emulsifying agents. U.S.D.A. Bur. Ent. and Plant Quar., Div. Insecticide Investigations Mimeo Pub. E-504.

Hartzell, A., and F. Wilcoxon. 1932. Some factors affecting the efficiency of contact insecticides. II. Chemical and toxicological studies of pyrethrum. Contrib. Boyce Thompson Inst. 4(1): 107–17.

King, H. L., and D. E. H. Frear. 1943. Relation of chemical constitution of some N-heterocyclic compounds to toxicity to *Tetranychus telarius* (L.). JOUR. ECON. ENT. 36(2): 263–5.

Richardson, C. H., and H. H. Shepard. 1930. The insecticidal action of some derivatives of pyridine piperidine and of some aliphatic amines. Jour. Agr. Res. 40(11): 1007–15.

Richardson, C. H., and C. R. Smith. 1923. Studies on contact insecticides. U.S.D.A. Bul. **1160**.

Tattersfield, F., and C. T. Gimingham. 1927. Studies on contact insecticides. V. The toxicity of the amines and N-heterocyclic compounds to *Aphis rumicis*, L. Ann. Appl. Biol. 14(2): 217–39.

Wibaut, J. P. 1934. Synthesis of nicotine derivatives. Natuurvw. Tijdschr. **16**: 106–11.

Reprinted December 30, 1944 from
JOURNAL OF ECONOMIC ENTOMOLOGY 37: (5):629

Mercury Substitutes for Cabbage Maggot Control[1]

L. E. DILLS, D. E. H. FREAR, and H. L. KING, *Pennsylvania Agricultural Experiment Station, State College*[2]

In the spring of 1943 the scarcity and high price of mercury emphasized the desirability of testing a number of mercury substitutes for control of the cabbage maggot, *Hylemia brassicae* (Bouché). Tar paper pads (Goff 1891, Slingerland 1894, Schoene 1914, Hodgkiss & Horsfall 1922), liberal applications of tobacco dust (O'Kane, Cleveland & Hadley 1923, Glasgow 1925) and screening (Schoene 1908, Gibson & Treherne 1916, Parrott & Glasgow 1917) also have been recommended. Under present conditions in Pennsylvania none of these appeared practical.

METHODS AND MATERIALS.—Since facilities were limited and it was necessary to test a considerable number of materials in a small space with some degree of accuracy, cabbage plants could not be used. Radishes offered the most practical solution of the problem, since they required little space and are preferred hosts of this insect. The most apparent disadvantage is that radishes are injured by some materials which may safely be used on cabbage.

Two field tests were conducted. In the first experiment white icicle radishes were used and the dust treatments were made when the first pair of primary leaves were developing. This was on May 19 and 20. The dichloroethyl ether was applied on May 23. Scarlet globe radishes were used in the second experiment. These radishes had been planted earlier than those in the first experiment. On May 24, when these treatments were made, there were two pairs of primary leaves with the third pair beginning to show.

Only one application was made, and with the exception of corrosive sublimate and dichloroethyl ether, all treatments were used as dusts. Calomel was prepared as a mechanical mixture with hydrated lime and with bentonite. All the remaining dusts were made by dissolving the toxicant, mixing with bentonite, evaporating to dryness, and grinding. In the first experiment the treatments were replicated six times, while in the second there were seven replications. The plots were 7 feet in length and were randomized to eliminate any differences in location. In addition to the replicated treatments, preliminary trials were made in the first experiment on eight other materials, using a reduced number of plots.

The dust was applied from an attachment to a plunger type 2-quart duster. This was made by attaching a 3-foot length of half-inch heavy rubber tubing to a glass tube which extended several inches through a large rubber stopper. Through another hole in the stopper was inserted a 7 mm. bent glass tube which was the discharge outlet. The dust to be used was put into a pint jar which the large rubber stopper fitted. The rubber tube was attached to the exit opening from the duster where the discharge pipe had been removed. This arrangement eliminated the problem of cleaning the duster and 2 persons could apply the treatments quite rapidly.

---

[1] Authorized for publication on June 15, 1944 as paper No. 1251 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Assistant Professor of Economic Entomology, Professor of Agricultural and Biological Chemistry and Instructor in Agricultural and Biological Chemistry (resigned March 1, 1944) respectively.

Results.—Since the per cent infestations of the treatments were not all between 20 and 80 per cent, the equivalent angle was used in the statistical treatment of the data.

Analysis of variance (Snedecor 1940) was made and is given in tables 1 and 2.

When applied early, a 10 per cent calomel dust was the best insecticide used which did not injure the radish plants. When it was applied late, as in the second experiment, it was found to be much less effective. With the same carrier there was not a significant difference between a 4 and a 10 per cent calomel dust but when a 4 per cent calomel dust with hydrated lime as the carrier was compared with a 10 per cent dust with bentonite the difference was significant. Calomel at 4 and at 10 per cent with bentonite as the carrier was more toxic than when used at the same strength with hydrated lime. When applied late, corrosive sublimate solution was found to be more toxic than 10 per cent calomel with hydrated lime as the carrier, but not significantly more toxic than 10 per cent calomel with bentonite as the carrier. The best insecticide tested late, which did not cause injury, was dichloroethyl ether.

Table 1.—Cabbage maggot tests on white icicle radishes.

(Experiment 1)

Treatment	Injury[1]	% Not Infested	Sum of Equivalent Angles
Calomel 10%	0	84.7	406.26
Paradichlorobenzene 10%	1	62.4	317.28
Paradichlorobenzene 20%	1	45.1	251.56
Paradichlorobenzene 40%	2	48.8	265.69
Dichloroethyl ether 1–400	0	74.8	362.14
Dichloroethyl ether 1-800	0	65.5	327.23
Naphthalene 10%	1	54.6	286.02
Naphthalene 20%	2	58.0	300.58
Naphthalene 40%	2	55.3	289.07
Check (no treatment)	0	50.1	270.69
2-ethyl-pyridine sulfate 10%	5	94.6	492.74
2-n-hexyl-pyridine sulfate 10%	3	74.3	374.39
2-mixed-undecyl-pyridine sulfate 10%	3	73.4	355.42
4-n-propyl-pyridine sulfate 10%	4	65.4	325.75
4-n-amyl-pyridine sulfate 10%	3	58.8	301.32
Quinoline sulfate 10%	2	67.4	332.71
Hexachlorethane 10%	1	58.2	298.62
Hexachlorethane 20%	2	47.0	259.59
Hexachlorethane 40%	2	49.8	269.19
Phenothiazine 10%	3	56.0	292.17
Phenothiazine 20%	4	58.8	301.70
Mathieson Alkali Weed Killer 10%	2	62.8	(2 plots)
Koppers Dormant Tar Oil 10%	5	100	(2 plots)
Beta naphthol 10%	D	—	(2 plots)
Pentacapryl laurylamine tripolyphosphate 10%	2	60.3	(2 plots)
Kerosene phosphine sulfide 10%	0	48.3	(2 plots)
Chlorophenyl thiophosphonic acid 10%	2	47.3	(2 plots)
Cresylic acid 10%	D	—	(4 plots)
Potassium sulfide 10%	1	68.1	(2 plots)

Analysis of variance on white icicle radishes

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F
Totals	125	20,076.65	—	—
Treatments	20	10,691.18	534.56	6.08**
Replicates	5	598.79	119.76	1.36
Experimental error	100	8,786.68	87.87	—

** Significant at the 1 per cent level.

Difference between sums of the equivalent angles necessary for significance at the 5 per cent level = 64.5.

[1] Injury: 0, none; 1, very slight; 2, slight; 3, moderate; 4 severe; 5, very severe; D, dead.

Table 2.—Cabbage maggot tests on scarlet globe radishes.

(Experiment 2)

Treatment	Injury[1]	% Not Infested	Sum of Equivalent Angles
Calomel 4% (bentonite)	0	56.9	343.37
Calomel 10% (bentonite)	0	65.9	381.22
Calomel 4% (hydrated lime)	0	44.6	293.33
Calomel 10% (hydrated lime)	0	50.6	317.35
Dichloroethyl ether 1–200	0	89.1	501.65
Dichloroethyl ether 1–400	0	80.5	447.98
Corrosive sublimate 1–1000	2	74.1	419.62
Check (no treatment)	0	41.7	281.28
Phenothiazine 40%	3	46.0	298.80
Naphthalene 40%	1	67.0	386.27
Paradichlorobenzene 40%	1	48.5	307.44
Hexachlorethane 40%	1	48.5	308.65
Koppers Dormant Tar Oil 5%	3	70.8	402.28
Calcium sulfide 10%	0	56.6	342.19

Analysis of variance.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Totals	97	12,047.81	—	—
Treatments	13	8,068.94	620.69	19.28**
Replicates	6	1,467.05	244.51	7.59**
Experimental error	78	2,511.82	32.20	—

** Significant at the 1 per cent level.

Difference between sums of the equivalent angles necessary for significance at the 5 per cent level = 42.3.

[1] Injury: 0, none; 1, very slight; 2, slight; 3, moderate.

The plots to which paradichlorobenzene, hexachlorethane, phenothiazine, 4-n-propyl-pyridine sulfate, 4-n-amyl-pyridine sulfate and quinoline sulfate were applied were not significantly better than those which had not been treated. Naphthalene at 40 per cent in the second experiment, 2-mixed-undecyl-pyridine sulfate, and calcium sulfide showed significant toxicity, but fell far short of giving satisfactory results.

Beta-naphthol and cresylic acid dusts, at the concentration used, killed the plants. Therefore no reliable information on toxicity to the insects was obtained. Limited trials of potassium sulfide, chlorophenyl thioposphonic acid, kerosene phosphine sulfide, pentacapryl laurylamine tripolyphosphate and *Mathieson Alkali Weed Killer* gave no indication that further tests should be conducted with these materials.

The 2-ethyl-pyridine sulfate was found to be very effective in preventing insect injury, but caused serious damage to radish plants. A limited trial of a dust containing 10 per cent *Koppers Dormant Tar Oil* gave similar results. In experiment 2, where the *Koppers Dormant Oil* was used at 5 per cent in replicated plots, it was found to have insecticidal value, but caused moderate plant injury. The 2-n-hexyl-pyridine sulfate also was found to be of value in reducing maggot damage, but unsafe to use on radishes. These three new materials, which had been found to be most effective in preventing maggot damage to radishes, were given preliminary tests on cabbage. Ten per cent dusts were applied to the dampened stems of late cabbage before setting. Records kept for more than 2 months did not indicate any harmful effects.

Summary.—Thirty-six different treatments, using 22 different materials, were applied to radishes under field conditions, and counts were made of cabbage maggot infestation. The new materials tested included several chlorinated hydrocarbons, N-heterocyclic compounds, and a number of miscellaneous substances. The best safe material tested early on radishes was a calomel-bentonite dust, while the best late treatment was with dichloroethyl ether. The smallest per cent infestation with maggot followed the use of a dormant tar oil and 2-ethyl-pyridine sulfate dust at 10 per cent, but injury to the radish plants was very severe. Several of the other pyridine derivatives were found to be quite effective, but caused considerable plant injury. Preliminary tests indicated that some of the treatments which caused serious injury to radishes, but were effective in preventing maggot damage can be used safely on cabbage plants. Further work is planned for the coming year. —6-23-44.

Literature Cited

Gibson, Arthur, and R. C. Trehorne. 1916. The cabbage root maggot and its control in Canada. Dominion of Canada. Dept. of Agr. Bul. **12.**

Glasgow, Hugh. 1925. Control of the cabbage maggot in the seedbed. New York, Agr. Expt. Sta. Bul. **512.**

Goff, E. S. 1891. A new preventive against the cabbage maggot. Eighth annual report of the Wisc. Agr. Expt. Sta., pp. 169–73.

Hodgkiss, H. E., and J. L. Horsfall. 1922. Protecting cabbage from the root maggot. Pennsylvania State College. Ext. Circ. **93.**

O'Kane, W. C., C. R. Cleveland and C. H. Hadley. 1923. Surface treatments for the cabbage maggot. New Hampshire Agr. Expt. Sta. Tech. Bul. **24.**

Parrott, P. J., and H. Glasgow. 1917. The radish maggot. New York Agr. Expt. Sta. Bul. **442.**

Schoene, W. J. 1908. Screening for the protection of cabbage seed beds. New York Agr. Expt. Sta. Bul. **301.**

Schoene, W. J. 1914. The cabbage maggot in relation to the growing of early cabbage. New York Agr. Expt. Sta. Bul. **382.**

Slingerland, M. V. 1894. The cabbage root maggot. Cornell Univ. Agr. Expt. Sta. Bul. **78.**

Snedecor, G. W. 1940. Statistical methods, 3 ed. Collegiate Press, Inc. Ames, Iowa.

Reprinted December 30, 1944 from
Journal of Economic Entomology 37: (5) :640

Copyright, 1944, by the Society for Experimental Biology and Medicine.
Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.
1944, 57, 124-125.

14723 P

Response of Rats to Diets Containing Propylene Glycol and Glycerol.

GAYLORD P. WHITLOCK, N. B. GUERRANT, AND R. ADAMS DUTCHER.
From the Department of Agricultural and Biological Chemistry, Pennsylvania State College.

The present paper is a brief preliminary report regarding some of the principal observations in connection with experiments which were started in 1941 in an attempt to compare the physiological effects of propylene glycol and glycerol when fed to rats at different levels of intake.

The first series of growth experiments covered a period of 20 weeks. The basal ration consisted, in parts per 100, of casein 20, salt mixture 4, Cell-U-Flour 2, butter fat 6, cod liver oil 2, brewer's yeast 6, and corn starch 60. Each experimental group consisted of 6 to 10 young rats. The basal ration was modified by replacing corn starch with glycerol and propylene glycol, respectively, at levels of 1, 3, 6, 10, 15, 20, 30, 40, 50, and 60 parts per 100 of total ration. Consequently, the first series of experiments comprised 21 dietary groups.

In a second series of experiments, groups of young rats were fed 4 basal diets identical with that of the first series with the exception that in 3 of these starch was replaced by glucose, sucrose, or dextrin, respectively. In this series, propylene glycol and glycerol were fed only at a 30% level, replacing, in each case, 30 parts of the carbohydrate.

A third series of feeding trials was conducted in which propylene glycol and glycerol were fed in isocaloric amounts at a level approximating 30% of the diet.

In the first series of experiments, no marked differences in growth response could be detected until the alcohols were fed at the 30% level. At this level the glycerol-fed animals grew at an approximately normal rate, while the propylene glycol animals grew less rapidly. Young rats, starting at 21 to 28 days of age, lost weight and died in a few days when the diet contained 40, 50, or 60% of propylene glycol. When young rats received diets containing comparable amounts of glycerol, growth was depressed and some animals eventually died but the majority of the animals survived the experimental period. Older (half grown) animals were able to tolerate the higher levels of propylene glycol but their growth responses were never equal to those of the animals receiving comparable amounts of glycerol.

When rats received propylene glycol and glycerol diets during alternate weeks (at the 30% level), growth responses were generally increased during glycerol periods and depressed during propylene glycol periods. While glycerol diets were consumed more rapidly because of palatability, calculations show that the glycerol-fed rats made greater gains per caloric unit of food intake. No differences could be noted when other carbohydrates replaced corn starch in the basal ration. Livers from glycerol-fed rats were invariably richer in glycogen than livers from rats receiving comparable amounts of propylene glycol.

A series of metabolism studies was also conducted in which groups of half-grown rats were fed comparable diets containing increasing amounts of glycerol or propylene glycol during alternate weeks. Without exception, rats excreted in their urine the greatest percentage of the ingested water, the greatest percentage of the thiamine intake, and the greatest percentage of the polyhydric alcohol intake during the weeks in which the propylene glycol-containing diets were fed. At the highest level of intake, more than 25% of the ingested propylene glycol was accounted for in the urine while in no instance was more than 1.5% of the ingested glycerol accounted for. This finding may partially explain an earlier observation that glycerol-fed rats made the greatest body weight gains per caloric unit of food intake.

While those groups of rats receiving the glycerol-containing diets gave birth to a greater number of litters and a greater number of young per litter, they were also more successful in weaning a higher percentage of the young than were similar groups of rats subsisting on comparable propylene glycol-containing diets. Reproduction and subsequent rearing of young were abnormally low with all groups of animals, including those on the purified basal ration. An attempt was made to correct this situation by addition of vitamin and mineral supplements without success. Consequently, further attempts to continue reproduction studies with purified diets were abandoned.

Reproduction studies with diets composed of the breeding colony diet (70%) and glycerol (30%) or propylene glycol (30%) resulted in some very significant differences. Rats which have received the glycerol-containing diet are now in the fifth generation, while second generation young have not yet been successfully weaned by the propylene glycol fed mothers. A more extensive series of diets which contain proteins, minerals, and vitamins from natural food sources and in which part of the energy is furnished as glycerol or as propylene glycol is now under investigation. A report on this phase of the investigation, together with the details of the studies herein reported, including pathological findings, will be forthcoming.

The concentrations of glycerol and propylene glycol in some of these diets were much greater than concentrations ordinarily ingested, but the information obtained may have a pertinent bearing on the relative toxicity of the two polyhydric alcohols.

Pa.-70

THE FERMENTATION OF CIGAR-LEAF TOBACCO AS INFLUENCED BY THE ADDITION OF YEAST

BY

M. F. GRIBBINS, D. E. HALEY and J. J. REID
(Contribution from Pennsylvania Agricultural Experiment Station)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. 69, No. 9 : : Washington, D. C., November 1, 1944
(Pages 373–381)

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

U. S. GOVERNMENT PRINTING OFFICE : 1944

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

P. N. ANNAND, CHAIRMAN
Chief, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration

JOHN W. ROBERTS
Principal Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration

BENJAMIN SCHWARTZ
Principal Zoologist, Chief, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

V. R. GARDNER
Director, Michigan Agricultural Experiment Station

C. B. HUTCHISON
Director, California Agricultural Experiment Station

W. H. MARTIN
Director, New Jersey Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to V. R. Gardner, Director, Michigan Agricultural Experiment Station, East Lansing, Mich., who is chairman of the Station subcommittee.

Published with the approval of the Director of the Budget. Issued on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal:	Domestic, $2.25 a year (2 volumes)
	Foreign, $3.75 a year (2 volumes)
Single numbers:	Domestic, 10 cents
	Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. When purchased in quantity of 100 or more, a reduction of 25 percent in the cost will be made. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

THE FERMENTATION OF CIGAR-LEAF TOBACCO AS INFLUENCED BY THE ADDITION OF YEAST[1]

By M. F. GRIBBINS, formerly *assistant in agricultural and biological chemistry*, D. E. HALEY, *professor of soils and phytochemistry*, and J. J. REID, *associate professor of bacteriology, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Tobacco of the cigar-leaf type must first undergo certain periods of fermentation before it can be used for cigar manufacture. The chemical changes that take place during fermentation involve, among others, a loss of some organic compounds and the synthesis of others with the final result that the aromatic and smoking qualities of the leaf are markedly improved.

Many theories have been advanced to explain the nature of the fermentation process. Among these may be mentioned the bacterial theory of Suchsland (*12*),[2] who claimed that certain micro-organisms have the power of acting upon leaf constituents and developing the characteristic flavor and aroma of fermented tobacco. The work of Behrens (*1*), Vernhout (*13*), and Koning (*4*) tends to support this theory.

Apparently, the first attempt to challenge the bacterial theory was made by Loew (*5*), who stated that leaf enzymes rather than bacteria provided the necessary mechanism of fermentation. For a long time this so-called enzyme theory of Loew's supplanted the bacterial theory. Recently, however, the work of Johnson (*2*) has revived the bacterial theory, and the later work of Reid and his coworkers (*6, 7, 8, 9, 10*) has proved conclusively that the major changes in fermentation are brought about through the activity of certain bacteria which usually develop in large numbers during the fermentation process, and that leaf enzymes play only a subordinate role.

The development of the micro-organisms of fermentation appears to be markedly affected by variations in the composition of the leaf. Some of these variations may occur during the curing process, but they may also be due in large measure to the environmental conditions under which the plants are grown, such as the nature of the soil, the season, and certain cultural practices which include the addition of fertilizers. In some cases the composition of the leaf may be of such a nature as to satisfy the nutrient requirements of undesirable rather than of desirable types of organisms. If, however, conditions are such as to favor the latter, the former do not appear

---

[1] Received for publication April 26, 1943. Paper No. 1151 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
[2] Italic numbers in parentheses refer to Literature Cited, p. 381.

Journal of Agricultural Research, Washington, D. C.
Vol. 69, No. 9
Nov. 1, 1944
Key No. Pa.-70

to thrive. It seems obvious, therefore, that a stimulation of the desirable types of fermentation organisms should aid materially in obtaining a satisfactory fermentation, and for this reason an effort was made as herein reported to obtain such a condition by the addition of a material high in growth-accessory substances, namely, yeast, and to study the effect of such a treatment upon the microflora of the leaves during the preliminary stages of fermentation. No direct action of yeast on the fermentation process was expected as Street (*11*) had shown that yeast cells live but a short time after they are applied to leaf surfaces.

The use of yeast as a supplementary treatment of cigar-leaf tobacco prior to fermentation was first introduced by Koller (*3*) in 1858, and later was employed by other European workers. Recently, Street (*11*) reported on the use of yeast in the fermentation of domestic cigar-leaf tobacco. His results showed that the temperature rises in the earlier stages of fermentation were greater with all yeast treatments than when water alone was used and, furthermore, that yeast treatment produced a product which appeared to be more completely fermented in a given period of time as judged by appearance and smoking qualities than untreated samples. In his investigations, however, he gave no direct consideration to the effect of the treatment on the microflora of the leaves.

PLAN OF THE EXPERIMENTS AND MATERIALS USED

Preliminary experiments to determine the effect of yeast treatment on the fermentation of Pennsylvania cigar-leaf tobacco were first undertaken on representative samples of the 1940 crop. At the conclusion of these experiments similar studies were conducted on representative samples of the 1941 crop. For this work representative samples of freshly cured tobacco were obtained from Landisville and from the tobacco experimental laboratory at Roseville, Pa., on December 31, 1941.

The 1941 crop had not received sufficient rainfall for normal development and hence may be termed a "dry-weather" crop. Tobacco produced during a dry season is usually far more difficult to ferment than that produced under conditions of normal and well-distributed rainfall. Moreover, the 1941 tobacco did not cure well owing to the dry weather which prevailed after the crop was harvested.

In preparing samples for fermentation, five wooden cases, each 30 inches long, 9 inches wide, and 11.5 inches deep were used. Each case held 40 pounds of tobacco under 400-pound pressure. The yeast, when used, was applied to the samples in the form of a 10 percent aqueous suspension before the tobacco was placed in the case, and at the rate of 0.75 percent of the solid content of the weight of the tobacco. This quantity had been found in previous experiments to have no deleterious effect on the aroma of the tobacco. The yeast used represented a moist, freshly prepared, commercially cultivated strain of *Saccharomyces cerevisiae* of the type used in bread baking.

EXPERIMENT 1

The treatments received by the tobacco both in the field and in the laboratory are presented in table 1. The tobacco in cases 4 and 5 came from five different experimental plots at the tobacco experimental laboratory, 8 pounds from each plot constituting the 40 pounds.

TABLE 1.—*Description of the tobacco samples used for fermentation studies (experiment 1)*

Sample No.	Case No.[1]	Where grown	Fertilizer treatment of crop		Supplementary laboratory treatment
			Pounds per acre	Formula	
1	1	Landisville	1,000	4-8-12	Control.
2	2	do	1,000	4-8-12	Yeast.
3	3	do	1,000	4-8-12	Do.
4	4	Roseville	1,000	3-9-24	Control.
5	5	do	1,000	3-9-24	Yeast.
6	4	do	1,000	3-9-12	Control.
7	5	do	1,000	3-9-12	Yeast.
8	4	do	1,000	3-6-12	Control.
9	5	do	1,000	3-6-12	Yeast.
10	4	do	1,000	3-9-18	Control.
11	5	do	1,000	3-9-18	Yeast.
12	4	do	1,000	3-12-12	Control.
13	5	do	1,000	3-12-12	Yeast.

[1] The tobacco used in cases 1 and 2 was from Kreider seed, that in case 3 from Kreider and Swarr seed mixed, and that in cases 4 and 5 from seed of a Swarr-Hibshman strain.

The tobacco was uniformly sprayed with water or yeast suspension and then exposed to the air for 24 hours. At the end of that period samples were taken for biological analysis. The results, representing the average of at least three samples in every case, are presented in table 2. In these and similar studies the bacteriological technique employed was that described by Reid, McKinstry, and Haley (*9*).

TABLE 2.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 1)*

Sample No.	Organisms per gram of tobacco			Nature and distribution of bacteria		
	Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Rods	
					Gram−	Gram+
	Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percen*
1	0.9	80	20	55	45	0
2	4.0	242	3,500	84	16	0
3	1.1	26	2,800	100	0	0
4	3.1	66	95	100	0	0
5	.2	34	1,300	0	50	50
6	.6	72	20	0	100	0
7	2.0	95	5,200	50	50	0
8	.1	50	30	0	100	0
9	7.0	57	13,200	---	---	---
10	.3	32	40	0	50	50
11	4.0	67	16,300	0	33	67
12	.7	72	260	0	50	50
13	3.0	30	7,900	33	33	33

When the tobacco was placed in the fermentation chamber, a thermometer was inserted in the center of each case. The centers of cases 4 and 5 were occupied by samples 8 and 9, respectively. The changes in temperature noted are presented in table 3. An effort was made to obtain an environmental temperature of 72° F. at first, but certain mechanical difficulties were experienced before this temperature could be attained and stabilized.

A humidity of 75 percent was maintained for the first 27 days of fermentation. At the end of that period the temperature was increased approximately 20° F., and the humidity was raised to 80 percent. On the 71st day of fermentation, the temperature was lowered to approximately 80° F. and the humidity to 75 percent.

On the 93d day the tobacco was removed from the cases and aerated. Samples were taken at that time from each case for obtaining the judgment of a leaf expert in respect to its quality and the extent of this preliminary phase of fermentation. The remainder was remoistened and an effort was made to approximate 32 percent moisture in all samples. The tobacco was then placed in the original cases and returned to the fermentation chamber.

TABLE 3.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 1)*

Interval (days)	Room temperature	Degrees above room temperature in—				
		Case 1	Case 2	Case 3	Case 4	Case 5
	°F.	*°F.*	*°F.*	*°F.*	*°F.*	*°F.*
1	75	7	9	6	6	15
2	68	13	20	14	5	16
3	72	7	14	12	6	18
4	72	6	16	15	6	18
5	72	6	15	16	6	18
6	72	6	15	16	8	17
7	72	6	14	17	10	18
8	72	6	13	17	11	18
9	72	6	12	17	11	18
10	72	6	11	16	13	16
12	73	6	8	14	15	15
14	71	6	8	13	16	14
16	71	6	7	11	14	12
18	72	4	5	7	11	9
20	72	4	4	6	7	6
25	71	4	4	3	5	7
30	92	1	3	3	5	6
40	89	4	4	3	5	7
50	89	4	3	6	5	8
60	90	3	2	4	4	5
70	91	2	1	3	4	4
80	80	1	1	2	4	3
90	80	0	1	1	3	3
100	80	6	6	2	9	6
110	80	5	6	2	6	4
120	80	5	5	2	4	4

When the tobacco was removed from the cases and reconditioned following the 92d day of fermentation, an opportunity was afforded for studying the microflora of the individual samples listed in table 1. The results obtained are presented in table 6.

TABLE 4.—*Moisture content of the tobacco samples before and during fermentation (experiment 1)*

Case No.	Moisture content after fermentation—interval (days)					
	0	12	19	54	70	92
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	29.3	29.2	29.2	29.0	30.7	27.3
2	35.0	33.4	32.1	35.1	33.7	32.9
3	29.2	28.1	27.0	32.8	31.3	30.1
4	22.2	22.1	22.0	23.8	26.0	25.5
5	22.4	22.3	22.2	24.3	28.8	28.9

TABLE 5.—*Number and kind of organisms on samples of tobacco taken from cases at different intervals during the fermentation period (experiment 1)*

Interval (days)	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms	Rods	
							Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
12	1	180	300	---	100	---	---	---
	2	2,390	500	1,000	100	---	---	---
	3	1,250	760	1,100	---	---	100	---
19	1	110	18	---	100	---	---	---
	2	2,400	49	1,000	100	---	---	---
	3	95	6,500	500	87	---	4	9
27	3	21	480	4,500	40	60	---	---
	4	4	285	2,200	44	28	---	28
	5	5.2	800	4,000	100	---	---	---
35	1	1,900	6	0	79	21	---	---
	2	1,500	3	22	79	21	---	---
	3	330	780	800	95	5	---	---
	4	4.6	25	110	90	10	---	---
	5	5.4	3,400	2,000	100	0	---	---
54	1	1,230	4	0	100	0	---	---
	2	410	.8	3	84	16	---	---
	3	820	37	12	84	16	---	---
	4	5	9	1,800	100	0	---	---
	5	700	270	40	89	11	---	---
70	1	995	7	60	91	9	---	---
	2	560	(1)	30	100	0	---	---
	3	1,100	6	3	95	5	---	---
	4	1,300	30	67	91	9	---	---
	5	2,110	21	680	95	5	---	---

[1] Trace.

Samples of tobacco were taken by means of borings made into the center of the cases as the fermentation progressed. Moisture determinations were made on these samples before they were placed in the case, and on the 12th, 19th, 54th, 70th, and 92d day of fermentation. The results obtained are given in table 4.

Samples removed from cases at different intervals were analyzed to determine the number of organisms and the nature of those predominating. The results are given in table 5.

TABLE 6.—*Number and kind of organisms on the leaves of the 13 original samples of tobacco at the end of the 92d day of fermentation*

Sample No.	Case No.	Supplementary treatment	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
			Million	*Thousand*	*Thousand*	*Percent*	*Percent*
1	1	Control	590	<1	<1	87	13
2	2	Yeast	720	1	<1	63	37
3	3	do	740	1	<1	93	7
4	4	Control	220	1	310	93	7
5	5	Yeast	930	1	<1	87	13
6	4	Control	1,060	1	28	87	13
7	5	Yeast	420	1	<1	80	20
8	4	Control	680	1	75	63	37
9	5	Yeast	510	1	<1	87	13
10	4	Control	1,380	1	570	87	13
11	5	Yeast	650	1	96	93	7
12	4	Control	670	1	107	100	0
13	5	Yeast	1,960	1	<1	80	20

Tests conducted prior to fermentation indicated a good burn in the tobacco making up cases 1, 2, and 3; the burn of the tobacco in cases 4 and 5 was classed as fair. In every instance, however, the aroma was classed as poor.

The samples of tobacco taken at the end of the 92d day of fermentation and submitted to the judgment of an expert on cigar-leaf tobacco yielded the results shown in table 7.

TABLE 7.—*Burning quality, aroma, and fermentation of samples of tobacco on the 92d day of fermentation, as judged by an expert on cigar-leaf tobacco*

Case	Burn	Aroma	Degree of fermentation
1	Good	Poor	Not pronounced.
2	do	Fair	Fair to good.
3	do	Very mild	Very good; best of all samples tested.
4	Fair	Poor	Not pronounced.
5	Good	Good	Good; second only to sample 3.

EXPERIMENT 2

At the conclusion of the first experiment a second and somewhat similar experiment was instituted. For this experiment two cases were filled with cured tobacco from the southern part of Lancaster County and two with tobacco from the laboratory at Roseville. The Roseville tobacco came from duplicates of the plots from which the tobacco used in cases 4 and 5 of experiment 1 had been obtained. All these samples had been kept in bales for 4 months longer than those of experiment 1.

An effort was made to approximate a 28-percent moisture content and to maintain a humidity of 75 percent and a temperature in the fermentation chamber of 80° F. The experiment was started on May 2, 1942. As in the first experiment, samples were taken for analysis after the yeast and moisture treatment and before the tobacco was placed in the cases.

A description of the samples is given in table 8 and the results of the initial study of leaf organisms are presented in table 9. The temperature changes as influenced by yeast treatment are shown in table 10.

TABLE 8.—*Description of the tobacco samples used for fermentation studies (experiment 2)*

Sample No.	Case No.	Fertilizer treatment of crop		Supplementary laboratory treatment
		Pounds per acre	Formula	
20	11	1,000	4- 8-12	Control.
21	12	1,000	4- 8-12	Yeast.
22	13	1,000	3- 9-18	Control.
23	14	1,000	3- 9-18	Yeast.
24	13	1,000	3-12-12	Control.
25	14	1,000	3-12-12	Yeast.
26	13	1,000	3- 9-24	Control.
27	14	1,000	3- 9-24	Yeast.
28	13	1,000	3- 6-12	Control.
29	14	1,000	3- 6-12	Yeast.
30	13	1,000	3- 9-12	Control.
31	14	1,000	3- 9-12	Yeast.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 2)*

Sample No.	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Coccoid forms	Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
20	11	0.8	91	1	83	17		
21	12	2.2	950	520	91	9		
22	13	.8	9	53	100			
23	14	7.7	110	4,900	100			
24	13	.7	540	470	80		10	10
25	14	.7	131	1,400	100			
26	13	1.2	25	760	90			10
27	14	1.3	410	3,200	100			
28	13	.3	13	15	100			
29	14	9.2	58	8,400	100			
30	13	.1	30	36	100			
31	14	.7	90	4,100	100			

TABLE 10.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 2)*

Interval (days)	Room temperature	Degrees above room temperature in—			
		Case 11	Case 12	Case 13[1]	Case 14[1]
	°F.	*°F.*	*°F.*	*°F.*	*°F.*
1	81	3	11	4	4
2	81	5	12	4	4
3	80	7	15	6	11
4	80	8	16	6	13
5	80	9	17	6	14
6	80	9	17	6	14
7	80	9	16	7	13
8	80	9	15	7	11
9	80	9	12	6	10
10	80	8	10	5	7
12	81	7	9	5	6
14	80	7	9	6	6
16	80	7	8	5	5
18	80	5	6	4	5
20	80	5	6	4	5
25	80	5	6	4	5
30	80	5	7	4	5
40	80	5	7	5	5
50	81	3	4	2	2
60	80	3	3	3	1
70	80	2	2	2	0
80	80	2	1	1	0
90	80	2	1	1	0
100	80	1	0	1	0
110	80	0	0	0	0
120	80	0	0	0	0

[1] The center of cases 13 and 14 was occupied by samples 26 and 27 respectively.

Samples were removed from the four cases on the 18th and 38th day of fermentation and were analyzed to determine the number of organisms and the nature of those predominating. The results are presented in table 11.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis cases at different intervals during the fermentation period (experiment 2)*

Interval (days)	Case No.	Moisture	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
		Percent	*Million*	*Thousand*	*Thousand*	*Percent*	*Percent*
18	11	24.2	2.4	68	<1	93	7
	12	25.9	600	7	8,400	87	13
	13	24.7	400	20	4,000	100	0
	14	28.0	2,700	27	5,100	93	7
38	11	25.0	1,270	<1	210	100	0
	12	28.1	2,900	13	8	100	0
	13	23.7	1,390	4	109	100	0
	14	30.8	1,790	<1	3	73	27

Tests conducted before fermentation showed that the tobacco making up cases 11 and 12 possessed a good burn but poor aroma while the tobacco making up cases 13 and 14 possessed a fair burn and poor aroma. Examination of these particular samples, after 120 days of fermentation, indicated an improvement in both burn and aroma as a result of the yeast treatment, paralleling the results obtained in experiment 1.

DISCUSSION OF RESULTS

The addition of yeast to cigar-leaf tobacco under the conditions of the experiments herein described was reflected in a relatively high initial increase in temperature during the preliminary stages of fermentation, as indicated in tables 3 and 10.

An examination of the samples receiving yeast showed the presence of a number of true yeasts 24 hours after treatment, but no other occasion of sampling was the presence of these organisms noted. On all samples, however, the presence of certain yeastlike organisms was observed, whether or not the samples were treated with yeast.

The number of these organisms was definitely increased as a result of yeast treatment, which appeared to be beneficial to the fermentation process. Entirely different yeastlike organisms were first noted on samples taken from the cases used in experiment 1 at the end of the 92d day of fermentation. In this instance, however, the addition of yeast, on the whole, appeared to be antagonistic to the development of these particular organisms.

The experiments to determine the influence of yeast treatment on the bacterial flora of the tobacco samples yielded somewhat erratic results. Owing to the differences ordinarily prevailing in the chemical composition of the leaves and the difficulties encountered in obtaining uniform samples, this was to be expected. On the whole, however, the addition of yeast resulted in an increase in the number of bacteria during the preliminary stages of fermentation. With but few exceptions, a high bacterial count was paralleled by a low mold content, which is considered highly desirable. There appeared to be little, if any, increase in mold development as a result of yeast treatment.

SUMMARY AND CONCLUSIONS

The results of fermentation studies dealing with the application of bakers' yeast to Pennsylvania cigar-leaf tobacco of the 1940 and 1941 crop are presented. The following conclusions seem justified:

(1) Initial temperature gains were greater with all yeast-treated tobacco under the conditions of these experiments.

(2) The addition of yeast stimulated the development of organisms considered necessary to the fermentation process.

(3) A more rapid initial fermentation was evidenced in the case of the yeast-treated samples.

LITERATURE CITED

(1) Behrens, J.
1896. DIE BEZIEHUNGEN DER MIKROORGANISMEN ZUM TABAKBAU UND ZUR TABAKFABRIKATION. Centbl. f. Bakt. [etc.] Abt. (II) 2:514–527, 540–545.
(2) Johnson, J.
1934. STUDIES ON THE FERMENTATION OF TOBACCO. Jour. Agr. Res. 49: 137–160, illus.
(3) Koller, J. B. C.
1858. DER TABAK IN NATURWISSENSCHAFTLICHER, LANDWIRTHSCHAFTLICHER U. TECHNISCHER BEZIEHUNG. 134 pp. Augsburg.
(4) Koning, C. J.
1900. DER TABAK; STUDIEN ÜBER SEINE KULTUR UND BIOLOGIE. 86 pp., illus. Amsterdam and Leipzig.
(5) Loew, O.
1899. CURING AND FERMENTATION OF CIGAR LEAF TOBACCO. U. S. Dept. Agr. Rpt. 59, 34 pp.
(6) McKinstry, D. W., Haley, D. E., and Reid, J. J.
1938. A BACTERIOLOGICAL STUDY OF THE BULK FERMENTATION OF CIGAR LEAF TOBACCO. (Abstract) Jour. Bact. 35: 71.
(7) Reid, J. J., Haley, D. E., McKinstry, D. W., and Surmatis, J. D.
1937. THE RELATION OF CATALASE ACTIVITY TO THE MICROFLORA OF CURED AND FERMENTING TOBACCO. (Abstract) Jour. Bact. 34: 460.
(8) ——— McKinstry, D. W., and Haley, D. E.
1937. THE FERMENTATION OF CIGAR LEAF TOBACCO Science 86: 404.
(9) ——— McKinstry, D. W., and Haley, D. E.
1938. STUDIES ON THE FERMENTATION OF TOBACCO. 1. THE MICROFLORA OF CURED AND FERMENTING CIGAR LEAF TOBACCO. Pa. Agr. Expt. Sta. Bul. 356, 18 pp., illus.
(10) ——— McKinstry, D. W., and Haley, D. E.
1938. STUDIES ON THE FERMENTATION OF TOBACCO. 2. MICROÖRGANISMS ISOLATED FROM CIGAR LEAF TOBACCO. Pa. Agr. Expt. Sta. Bul. 363, 18 pp., illus.
(11) Street, O. E.
1938 THE ROLE OF YEASTS IN THE FERMENTATION OF TOBACCO. 1. PRELIMINARY STUDIES. Conn. Agr. Expt. Sta. Bul. 410, pp. 414–443, illus.
(12) Suchsland, E.
1891. UEBER TABAKSFERMENTATION. Deut. Bot. Gesell. Ber. 9: 79–81.
(13) Vernhout, J. H.
1899. ONDERZOEK OVER BACTERIËN BIJ DE FERMENTATIE DER TABAK. Buitenzorg Meded. s' Lands Plantentuin 34, 49 pp., illus.

○

Reprint from POULTRY SCIENCE: Vol. XXIII, No. 6
November, 1944

Lactalbumin as a Protein Supplement for Growing Chickens[1]

WALTHER H. OTT[2] AND R. V. BOUCHER
Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pennsylvania

(Received for publication April 13, 1944)

INFORMATION gained from chick feeding trials with various protein concentrates is of immediate practical importance to poultrymen. Knowledge of the relative growth-promoting properties of the commercial protein materials, individually as well as in combination, is useful in formulating the most economical and efficient ration under any particular set of conditions.

From time to time there become available commercially new high-protein products which may be useful in poultry feeding. Actual feeding tests under controlled conditions are desirable before acceptance or rejection of such products by poultrymen.

A commercial grade of lactalbumin is now available which has a protein content of about 40 percent and an ash content of about 30 percent. This protein concentrate would be useful in poultry rations if it should prove to contain protein of good quality and mineral content that is not harmful.

Lactalbumin itself has been recognized as a protein of high nutritive value since the extensive early investigations by Osborne and Mendel (1919 and 1924). Kik (1938) recently reported that lactalbumin has a higher biological value than casein. Nevertheless, the practical value of commercial lactalbumin in poultry rations can be best determined by feeding trials with poultry.

EXPERIMENTAL

This paper is a report of investigations on the growth promoting properties of commercial grade lactalbumin in different combinations with meat scraps and with soybean oil meal. Lactalbumin is a by-product of the manufacture of beta lactose and is prepared by heat coagulation (pH 6.7 at 180 to 195°F.) from whey following hydrochloric acid precipitation of the casein (pH 4.2) from the milk. Meat scraps was chosen because it is one of the most widely used animal protein concentrates in poultry rations, and soybean oil meal because it is one of the most important plant protein concentrates, becoming steadily more abundant.

The objects were to determine (1) if the lactalbumin could be used in starting rations, (2) if a supplementary relationship existed between lactalbumin and meat scraps or soybean oil meal, and, if so, (3) the ratio-promoting maximum growth.

Relative growth of chicks to eight weeks of age was taken as the criterion for comparison. Rations containing 21 percent protein were used to obtain data under conditions of maximum growth. Since more economical growth is usually obtained by the commercial poultrymen with lower levels of protein, one experiment was conducted with diets containing 18 percent protein.

---

[1] Authorized for publication as paper No. 1232 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Now with the Merck Institute for Therapeutic Research, Rahway, New Jersey.

In each experiment, groups of chicks selected for uniformity of body weight were wing-banded, placed in electrically heated, wire starting batteries and fed the experimental diets to eight weeks of age. The chicks were individually weighed at seven-day intervals. Management and other details were kept as uniform as possible from group to group.

A commercial type of all-mash starting ration modified to contain one source of animal protein was selected for this work. Five percent brewers' dried yeast was included to insure that each experimental diet contained sufficient amounts of the water-soluble vitamins, regardless of the quantities of lactalbumin, meat scraps or soybean oil meal present. A second reason for inclusion of yeast in the basal diet was that it is a good source of choline which is a nutritive essential for chickens and for some species is required for normal metabolism of the sulfur containing amino acids present in the protein concentrates under investigation (Mulford and Griffith, 1942). Eight ingredients of this ration, which were kept the same in all diets, constituted the following basal mixture:

Basal Mixture

Wheat bran	15	lbs.
Wheat middlings	15	lbs.
Ground oats	10	lbs.
Alfalfa meal	7.5	lbs.
Brewers' dried yeast	5	lbs.
400-D fish liver oil	0.5	lbs.
Sodium chloride	0.5	lbs.
Manganese sulfate	0.014	lbs.
Total	53.514	lbs.

The remaining 46.5 pounds of each experimental diet consisted of ground yellow corn and meat scraps or lactalbumin, or different combinations of meat scraps and lactalbumin. Soybean oil meal was used in place of meat scraps in the tests for supplementary action between soybean oil meal and lactalbumin. The protein level in all diets in each experiment was kept as uniform as possible.

A. *Lactalbumin and Meat Scraps*

Experiment 1.—In the first experiment lactalbumin replaced meat scraps, unit weight for unit weight, in the diets. Relatively uniform protein and mineral relationships were maintained without adjustment of other constituents of the diet, due to the similar composition of these products, as indicated by the following percentage values which were determined by chemical analyses:

	Protein	Ash	Calcium	Phosphorus
Lactalbumin	46.64	28.56	9.98	2.74
Meat scraps	44.28	28.76	9.81	2.60

It will be noted that the meat scraps was particularly low in protein, notwithstanding the factthat it was a standard commercial product, normal in appearance and odor, bought on the local market and carrying a minimum protein guarantee of fifty percent.

The five diets, listed in Table 1, were fed to Single Comb White Leghorn chicks from one day to eight weeks of age. Critical examination of the complete growth curves of the groups of chicks in this and the following experiments showed that the eight-week body weights indicated accurately the relative performance on the different diets. Accordingly, the mean weights at eight weeks of age are reported in each experiment.

Growth data in experiment 1 (Table 2) indicated that the protein from lactalbumin was better utilized by males than the protein from meat scraps. The occurrence of highest growth on diet 4 with both sexes suggests mutually supplementary action between lactalbumin and meat scraps. This supplementary action seemed to be greatest with a relatively high proportion (76 percent) of lactalbumin protein.

TABLE 1.—*Composition on a percentage basis of diets in experiment 1*

Ingredient	Diet 1	2	3	4	5
Basal mixture	53.5	53.5	53.5	53.5	53.5
Ground yellow corn	26.5	26.5	26.5	26.5	26.5
Lactalbumin	0.0	5.0	10.0	15.0	20.0
Meat scraps	20.0	15.0	10.0	5.0	0.0
Riboflavin	0.0004	0.0004	0.0004	0.0004	0.0004
Total Protein (calc.)	20.8	20.9	21.0	21.1	21.2
Protein supplied by Lactalbumin and Meat scraps	8.9	9.0	9.1	9.2	9.3
Protein Ratio* $\frac{L}{MS}$	$\frac{0}{100}$	$\frac{26}{74}$	$\frac{51}{49}$	$\frac{76}{24}$	$\frac{100}{0}$

* Ratio of lactalbumin protein (L) to meat scraps protein (MS).

Higher efficiency of feed utilization with increase in body weight is indicated in Table 2. The values are given in terms of weight of feed per unit weight of gain. The high rate of growth on diet 4 was made with the highest feed efficiency. The data indicate that the increased growth rate resulted from higher feed efficiency rather than from proportionately higher feed intake.

TABLE 2.—*Mean body weights (±S.E.)* at eight weeks of age and feed efficiencies of Single Comb White Leghorns in experiment 1*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
1	9	592.4±27.1	6	493.2±15.8	4.32
2	9	600.2±37.7	6	560.5±14.6	4.55
3	7	616.3±28.7	8	577.0±18.2	4.34
4	7	652.1±27.2	8	591.6±10.4	4.19
5	9	614.4±39.5	6	493.5±13.3	4.28

* S.E. is abbreviation for standard error.

Experiment 2.—In this experiment Barred Rock chicks were used to determine whether or not this breed would respond the same as the White Leghorns. The lot of lactalbumin available for this and the following experiments was materially lower in protein content than the first sample. Meat scraps, purchased on the local market like that used in experiment 1, was higher in protein content as indicated by the following comparative analyses expressed as percent:

	Protein	Ash	Calcium	Phosphorus
Lactalbumin	40.25	28.98	9.85	4.05
Meat scraps	49.56	27.98	9.34	4.57

Because of the difference in protein content between meat scraps and lactalbumin, these concentrates were included in the diets in such quantities as to supply a total of nine pounds of crude protein per 100 pounds of diet. The various combinations were made up on the basis of unit weight of protein rather than unit weight of concentrate.

Table 3.—*Composition on a percentage basis of diets in experiment 2*

Ingredient	Diet 6	7	8	9	10
Basal mixture	53.5	53.5	53.5	53.5	53.5
Ground yellow corn	28.34	27.29	26.24	25.19	24.14
Lactalbumin	0.0	5.59	11.18	16.77	22.36
Meat scraps	18.16	13.62	9.08	4.54	0.0
Total Protein (Chem. anal.)	20.75	21.13	20.94	20.69	20.63
Protein Ratio $\frac{L}{MS}$	$\frac{0}{100}$	$\frac{25}{75}$	$\frac{50}{50}$	$\frac{75}{25}$	$\frac{100}{0}$

Five diets similar to those used in experiment 1 were prepared according to the formulae in Table 3 and fed to Barred Rock chicks from day-old to eight weeks of age.

Table 4.—*Mean body weights (±S.E.) at eight weeks of age and feed efficiencies of Barred Rocks in experiment 2*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
6	7	754.7±72.6	6	675.8±34.3	2.73
7	6	836.2±59.2	7	737.1±25.0	2.55
8	11	872.4±34.5	3	721.7±35.4	2.75
9	7	927.6±42.2	8	736.5±33.8	2.48
10	6	846.8±39.5	9	729.8±23.4	2.60

Results in this experiment, using Barred Rocks, checked quite closely the results in the first experiment, in which White Leghorns were used. Growth of both sexes of Barred Rocks was higher with lactalbumin (diet 10) than with meat scraps (diet 6) as the only protein concentrate in the diet. The mutually supplementary action between lactalbumin and meat scraps is again evident, especially with the males. Growth of the males in particular was highest with the diet having a high proportion of lactalbumin (diet 9).

Data on feed efficiency show that the lactalbumin diet (10) was utilized more efficiently than the meat scraps diet (6). The still higher efficiency on two of the three intermediate diets is further evidence for mutually supplementary action between lactalbumin and meat scraps.

Table 5.—*Composition on a percentage basis of diets in experiment 3*

Ingredient	Diet 14	15	9	16	17	18	19	10
Basal mixture	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Ground yellow corn	25.61	25.40	25.19	24.98	24.77	24.56	24.35	24.14
Lactalbumin	14.53	15.65	16.77	17.89	19.01	20.12	21.24	22.36
Meat scraps	6.36	5.45	4.54	3.63	2.72	1.82	0.91	0.0
Total Protein (Chem. anal.)	21.44	21.25	21.38	21.06	21.31	21.25	21.13	21.31
Protein Ratio $\frac{L}{MS}$	$\frac{65}{35}$	$\frac{70}{30}$	$\frac{75}{25}$	$\frac{80}{20}$	$\frac{85}{15}$	$\frac{90}{10}$	$\frac{95}{5}$	$\frac{100}{0}$

Experiment 3.—In this trial the object was to determine more accurately the lactalbumin-meat scraps ratio giving the maximum growth. The lactalbumin and meat scraps were taken from the same lots previously used in experiment 2.

Eight diets were prepared in which the ratio of lactalbumin protein to meat scraps protein varied uniformly over the narrower range from 65:35 to 100:0 as shown in Table 5. Evidence in the previous experiments indicated that the highest growth rate could probably be obtained within this range.

Sexed Barred Rock chicks were used in order to insure nearly equal sex distribution in each group. All chicks were fed diet 6 until one week old, when eight groups of uniform weights were selected for the experimental period. Feeding of the test diets was begun the eighth day of age.

In view of the previous results, a curvilinear relationship would be expected to exist between the percent lactalbumin protein and the eight week body weights.

Table 6.—*Mean body weights (±S.E.) at eight weeks of age and feed efficiencies of Barred Rocks in experiment 3*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
14	9	786.8±31.6	7	719.4±34.8	2.68
15	8	777.1±50.8	8	767.5±20.8	2.63
9	9	827.8±31.1	7	799.0±45.5	2.57
16	7	898.4±41.4	9	777.7±21.8	2.40
17	9	824.7±36.7	7	769.0±23.9	2.62
18	7	900.3±49.4	9	755.4±27.1	2.49
19	7	711.8±36.0	8	714.8±19.2	2.71
10	9	797.8±32.7	7	750.3±23.7	2.72

With the males this relation is evident but the curve is rather irregular, as indicated by the data in Table 6. Highest growth was promoted by lactalbumin protein to meat scraps protein ratios of 80:20 (diet 16) and 90:10 (diet 18).

With the females the data clearly indicate that the 75:25 ratio (diet 9) promoted the highest rate of growth. This level of lactalbumin is slightly lower than that giving the maximum response with the males. There is also some evidence in the two previous experiments that the females utilized a lower level of lactal-

Table 7.—*Composition on a percentage basis of diets in experiment 4*

Ingredient	Diet 29	30	31	32	33	34	35	36
Basal mixture	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Ground yellow corn	38.41	37.79	37.47	37.15	36.84	36.53	36.21	35.90
Lactalbumin	0.0	2.64	3.97	5.29	6.61	7.93	9.26	10.58
Meat scraps	8.07	6.05	5.04	4.04	3.03	2.02	1.01	0.0
Total Protein (Chem. anal.)	18.50	18.19	18.13	18.19	17.88	18.13	18.13	17.63
Protein Ratio $\frac{L}{MS}$	$\frac{0}{100}$	$\frac{25}{75}$	$\frac{37.5}{62.5}$	$\frac{50}{50}$	$\frac{62.5}{37.5}$	$\frac{75}{25}$	$\frac{87.5}{12.5}$	$\frac{100}{0}$

TABLE 8.—*Mean body weights (±S.E.) at eight weeks of age and feed efficiencies of Barred Rocks in experiment 4*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
29	10	572.8±16.3	7	503.3±16.6	2.86
30	10	558.1±18.7	7	564.0±26.4	2.74
31	7	667.6±22.3	10	575.4±27.7	2.68
32	9	699.0±19.7	8	614.5±18.9	2.68
33	9	735.8±22.2	8	658.0±21.0	2.63
34	8	754.9±29.2	9	626.8±27.6	2.59
35	7	726.9±35.2	10	590.1±21.2	2.64
36	8	715.0±29.3	9	575.7±19.1	2.71

bumin proportionately better than did the males. Feed efficiency was observed to be highest with a high proportion of lactalbumin in combination with at least 10 parts of meat scraps protein.

Experiment 4.—Diets containing about 18 percent protein were used in this experiment to determine relative performance at a submaximum rate of growth. To modify the basal diet accordingly, it was necessary to reduce the animal protein to four percent of the ration which is nearer the quantity of animal protein found in commercial mashes.

The composition of the eight diets used in this trial is given in Table 7. The lactalbumin protein to meat scraps protein ratios were the same as those used in experiment 2 with the addition of three intermediate ratios. A new lot of lactalbumin, used in this experiment, had the following percentage composition by chemical analyses:

Protein	Ash	Calcium	Phosphorus
37.81	33.28	10.23	3.81

Barred Rock chicks were prepared for this trial in the same manner as those used in experiment 3. Feeding the test diets was begun when the chicks were one week old.

The relative growth-promoting properties of lactalbumin, meat scraps, and the various combinations were essentially the same with the 18 percent protein level (Table 8) as with 21 percent protein in the diet. Lactalbumin promoted higher growth with both sexes and more efficient growth than did meat scraps. The various combinations produced results indicative of mutually supplementary action, which was greatest when lactalbumin supplied 62.5 to 75 percent of the animal protein.

Evidence was again obtained that maximum growth with males occurred on a higher percentage of lactalbumin than did the maximum growth with the females. Highest growth of the males occurred with a lactalbumin protein to meat scraps protein ratio of 75:25 (diet 34). The lower ratio of 62.5:37.5 (diet 33) promoted maximum growth of the females. Growth on these two ratios was more efficient than with any other combination, which agrees closely with the results in the previous experiments.

TABLE 9.—*Composition on a percentage basis of diets in Experiment 5*

Ingredient	Diet 11	12	13	10
Basal mixture	53.5	53.5	53.5	53.5
Ground yellow corn	21.93	23.15	24.44	24.14
Lactalbumin	5.59	11.18	16.77	22.36
Soybean oil meal	15.87	10.58	5.29	0.0
Steamed bonemeal	1.73	.90	0.0	0.0
Ground limestone	1.38	.69	0.0	0.0
Total Protein (Chem. anal.)	20.88	20.75	20.75	20.63
Protein Ratio $\frac{\text{L*}}{\text{SM}}$	$\frac{25}{75}$	$\frac{50}{50}$	$\frac{75}{25}$	$\frac{100}{0}$

* Ratio of lactalbumin protein (L) to soybean oil meal protein (SM).

TABLE 10.—*Mean body weights (±S.E.) at eight weeks of age and feed efficiencies of Barred Rocks in experiment 5*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
11	8	758.1±41.7	6	799.5±31.3	2.72
12	9	816.2±63.8	6	755.8±21.9	2.67
13	7	812.9±20.0	6	762.2±37.2	2.71
10	6	846.8±39.5	9	729.8±23.4	2.60

B. *Lactalbumin and Soybean Oil Meal*

Experiment 5.—Three diets containing different combinations of lactalbumin and soybean oil meal were fed to Barred Rocks to compare the growth promoting properties of these two protein sources. The percentage composition of the expeller process soybean oil meal used in this and the following experiment was as follows:

Protein	Ash	Calcium	Phosphorus
42.50	5.35	0.36	0.62

The groups in experiment 5 were run concurrently with the groups in experiment 2. Diet 10, of course, was represented by the same lot of chicks for both experiments. Steamed bonemeal and ground limestone were used to maintain uniform calcium and phosphorus levels in the diets containing soybean oil meal (Table 9).

An interesting result of this trial was the apparently opposite growth responses of the two sexes, as indicated in Table 10. Weights of the males increased with the increasing proportions of lactalbumin in the diets. With the females, however, growth appeared to decrease with increasing amounts of lactalbumin. The almost

TABLE 11.—*Composition on a percentage basis of diets in experiment 6*

Ingredient	Diet 20	21	22	23	24	25	26	27	28
Basal mixture	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5
Ground yellow corn	19.14	19.50	20.22	20.94	21.66	22.39	23.11	23.83	24.20
Lactalbumin	1.40	2.79	5.59	8.38	11.18	13.97	16.77	19.56	20.96
Soybean oil meal	19.85	18.53	15.88	13.24	10.59	7.94	5.29	2.65	1.32
Steamed bonemeal	5.07	4.72	4.00	3.27	2.53	1.81	1.09	0.37	0.0
Ground limestone	1.02	0.94	0.79	0.65	0.52	0.37	0.22	0.07	0.0
Total Protein (Chem. anal.)	23.25	23.19	23.19	23.06	22.88	22.94	22.88	22.38	22.25
Protein Ratio $\frac{\text{L}}{\cancel{\text{MS}}\ \text{Sm}}$	$\frac{6.25}{93.75}$	$\frac{12.5}{87.5}$	$\frac{25}{75}$	$\frac{37.5}{62.5}$	$\frac{50}{50}$	$\frac{62.5}{37.5}$	$\frac{75}{25}$	$\frac{87.5}{12.5}$	$\frac{93.75}{6.25}$

TABLE 12.—*Mean body weights (±S.E.) at eight weeks of age and feed efficiencies of Barred Rocks in experiment 6*

Diet	Body weight				Feed efficiency
	Males		Females		
	No.	Grams	No.	Grams	Feed/gain
20	7	712.4±24.4	8	618.2±24.5	2.84
21	9	748.0±30.0	6	615.0±48.0	2.95
22	7	796.3±41.8	7	635.7±36.5	2.84
23	8	790.4±22.0	8	697.2±32.3	2.84
24	7	795.9±29.7	9	647.3±13.9	2.69
25	8	782.0 ~~762.0~~±54.6	7	677.4±33.4	2.67
26	8	828.4±38.6	8	709.5±23.2	2.78
27	8	852.4±36.8	8	708.2±20.7	2.73
28	8	849.8±40.6	8	698.6±21.6	2.61

linear relation between body weight and percentage of lactalbumin in the diet indicated the absence of appreciable mutually supplementary action between lactalbumin and soybean oil meal. Feed efficiency tended to increase with increasing amounts of lactalbumin.

Experiment 6.—Nine different combinations of lactalbumin and soybean oil meal were used in this trial. As indicated in Table 11, the ratio between lactalbumin protein and soybean oil meal protein was varied gradually throughout the range from 6.25:93.75 to 93.75:6.25. With these diets the results were expected to indicate more clearly the relation between these two proteins than was obtained in the previous experiment.

Body weight in this trial (Table 12) was highest with both sexes on the diets containing the higher amounts of lactalbumin. The nearly linear relationship between body weight and lactalbumin level again as in experiment 5 indicated the probable absence of mutually supplementary action between these two proteins. Growth on the highest level of lactalbumin was more efficient than on the highest level of soybean oil meal. Feed efficiency tended to show a linear relationship with the amount of lactalbumin in the diet. These results are further evidence that no mutually supplementary action exists between lactalbumin and soybean oil meal. However, lactalbumin supported a higher rate of growth than soybean oil meal.

C. *Incidence of Perosis*

Statistics on the incidence of perosis in the six experiments previously described are summarized in Table 13. In the tests with lactalbumin and meat scraps, 2.4 percent of the chicks were affected. Half of these were mild cases. In the tests with lactalbumin and soybean oil meal, 3.3 percent of the chicks developed perosis, one-third of which were mild cases.

TABLE 13.—*Number of cases of perosis in experiments with lactalbumin and meat scraps or soybean oil meal*

Experiment	Percent lactalbumin protein*						
	0	25	50	62.5	75	95	100
	No.	No.	No.	No.	No.	No.	No.
1, 2, 3, 4 (Meat scraps)	2	1	1	—	2	1	3
5, 6 (Soybean oil meal)	—	3	1	1	1	—	—

* Percent lactalbumin protein of the total protein in lactalbumin and meat scraps or soybean oil meal in the diet.

In all other respects the chicks were normal and vigorous, presenting a healthy appearance which included good feathering and pigmentation that did not vary from group to group. Droppings were normal in appearance in all groups.

DISCUSSION

The experimental work with lactalbumin and meat scraps has shown that lactalbumin promoted slightly greater growth in the chicks than did meat scraps when each was the only source of animal protein in the diet. Furthermore, less feed was required to produce one pound of gain on the lactalbumin diets than on the meat scraps diets. From these facts it would seem that the use of lactalbumin as a source of animal protein in poultry rations is nutritionally sound.

The results have clearly indicated a mutually supplementary action between these two protein concentrates. Both growth and feed efficiency tended to be materially higher when the two proteins were present in a diet than would be expected if no supplementary relation existed. Growth and feed efficiency were highest when lactalbumin supplied approximately 75 percent of the total protein furnished by lactalbumin and meat scraps in the diet.

The maximum supplementary effect was observed with a slightly higher proportion of lactalbumin for the males than for the females. This differential response may have been due to the difference in growth rate between the sexes. A possible explanation that suggests itself is that the more rapidly growing males probably have a higher requirement for some essential amino acid which may be found more abundantly in lactalbumin than in meat scraps. This suggestion is strengthened by the fact that the five percent brewers' yeast contained in all of the experimental diets, along with the contributions made by the other constituents of the diets, likely supplied optimum quantities of all the known as well as unknown members of the vitamin B complex. The superior response of males confirms the results of Irwin and Kempster (1942) that males utilize additional protein more efficiently than females.

Presumably a ratio of 75 parts lactalbumin protein to 25 parts meat scraps protein would be expected to give close to the maximum growth attainable by a combination of these two protein concentrates. In 100 pounds of a 21 to 22 percent protein starting ration, this ratio would be supplied by about 17 pounds of lactalbumin (40 percent protein) and 4.5 pounds of meat scraps (50 percent protein). In an 18 percent protein ration the amounts required would be about 8 pounds of lactalbumin and 2 pounds of meat scraps.

The experiments with lactalbumin and soybean oil meal strongly indicated that no mutually supplementary action exists between these two proteins. Evidently the growth response on a diet containing lactalbumin cannot be increased by the addition of soybean oil meal to the diet. The reverse, however, is true for the addition of relatively small amounts of lactalbumin to diets high in soybean oil meal increased the rate of growth.

Although perosis occurred in these experiments, the incidence was low. The fairly uniform distribution of the cases, regardless of the percentage of lactalbumin, soybean oil meal or meat scraps in the diet, is strong evidence that the protein supplements did not influence the development of perosis.

SUMMARY

1. In experiments with 75 Single Comb White Leghorns and 514 Barred Rocks, more rapid and efficient growth to eight weeks of age was obtained with lactalbumin than with meat scraps or soybean oil meal as the sole protein concentrate in an all-mash commercial type ration.

2. Mutually supplementary action was shown to exist between lactalbumin and meat scraps. Lactalbumin additions supplemented diets high in soybean oil meal but additions of soybean oil meal to diets high in lactalbumin neither increased rate of growth nor feed efficiency.

ACKNOWLEDGMENT

The authors wish to thank the Department of Poultry Husbandry for the chicks and the Smith, Kline and French Laboratories, Philadelphia, for the lactalbumin used in these experiments.

REFERENCES

Irwin, M. R., and H. L. Kempster. 1942. The value of soybean oil meal in broiler rations. Mo. Agr. Expt. Sta. Bull. 441, 16 pp.

Kik, M. C. 1938. Digestibility, metabolism and nutritive value of lactalbumin. Ark. Agr. Expt. Sta. Bull. 352, 24 pp.

Mulford, D. J., and W. H. Griffith. 1942. Choline metabolism. VIII. The relation of cystine and of methionine to the requirement of choline in young rats. J. Nutrition 23:91–100.

Osborne, T. B., and L. B. Mendel. 1924. The nutritive value of lactalbumin. J. Biol. Chem. 59:339–345.

Osborne, T. B., L. B. Mendel and E. L. Ferry. 1919. A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. 37:223–229.

STUDIES ON THE TOXICITY OF GOLD COMPOUNDS IN RATS

CHARLES W. DENKO, PH.D.
and
ARTHUR K. ANDERSON, PH.D.
State College, Pa.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College

Reprinted from
THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE
St. Louis

Vol. 29, No. 11, Pages 1168-1176, November, 1944

(Printed in the U. S. A.)

STUDIES ON THE TOXICITY OF GOLD COMPOUNDS IN RATS

Charles W. Denko, Ph.D.,* and Arthur K. Anderson, Ph.D.
State College, Pa.

THE reports of Møllgaard,[1] Forestier,[2] Towle and Grund[3] Hartfall, Garland, and Goldie,[4] Sabin and Warren,[5, 6] and others have created considerable interest in the use of gold compounds for the treatment of tuberculosis, lupus erythematosus, and arthritis. However, the frequent, varied toxic reactions and occasional fatalities in patients receiving gold drugs have been a hindrance to their extensive use. In an effort to find a nontoxic, therapeutically effective drug, investigators have tested a large number of gold preparations without discovering one that is completely satisfactory.

In 1940 Sabin and Warren[6] studied the effectiveness of gold compounds in curing an experimental mouse arthritis produced by inoculating mice with a pleuropneumonia-like organism. In their work they studied the toxicity of several gold compounds, using mice as test animals and using survival or death of the animal as an index of toxicity. Obviously this method gives no measure of the degree of toxicity in surviving animals.

In using a similar technique to test the toxicity of gold compounds in this laboratory, it was noted that mice receiving sublethal doses of gold salts exhibited an elevation of the nonprotein nitrogen level of the blood, often to two or three times normal values. From this it appeared that the nonprotein nitrogen value of the blood might be a better index of toxicity than survival or death. However, since a mouse had to be sacrificed to obtain sufficient blood for a nonprotein nitrogen determination, this method could hardly be considered satisfactory since it is often desirable to give an animal several doses of the drug. In order to eliminate the necessity of sacrificing an animal whenever its blood was to be analyzed, rats were used in the experiments reported here.

In the following report several gold compounds, some commercially available and others especially synthesized for this work, have been studied in order to obtain information with regard to their toxic reactions.

GOLD COMPOUNDS USED

Table I lists the gold compounds used in the following experiments together with their formulas and theoretical gold content. Especially prepared gold

---

*Present address: Research Laboratories, S. M. A. Corporation, Chagrin Falls, Ohio.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College.

This paper is taken from a thesis presented to the Graduate School of the Pennsylvania State College by Charles W. Denko in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

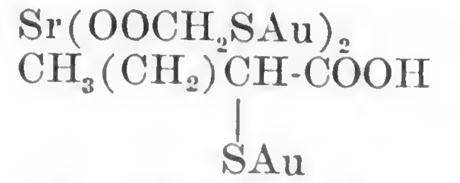
Authorized for publication July 18, 1944, as paper No. 1239 in the journal series of the Pennsylvania Agricultural Experiment Station.

Received for publication, Aug. 5, 1944.

compounds were analyzed for gold as an index of purity. These values are given together with the method used for the preparation of each prepared compound.

TABLE I

GOLD COMPOUNDS USED

NAME	FORMULA	PER CENT OF GOLD		METHOD OF PREPARATION
		THEORETICAL	FOUND§	
1. Aurothioglucose*	$C_6H_{11}O_5SAu$	50.		
2. Gold sodium thiosulfate†	$Na_3Au(S_2O_3)_2 \cdot 2H_2O$	37.4		
3. Colloidal gold sol‡				
4. Strontium aurothioglycolate	$Sr(OOCH_2SAu)_2$	59.56	60.61	(7), (8)
5. *α*-aurothio-*n*-butyric acid	 $\underset{\displaystyle SAu}{CH_3(CH_2)CH\text{-}COOH}$	62.24	61.40	(7), (8)
6. Auro-*n*-propyl xanthate	$CH_3(CH_2)_2OCSSAu$	59.29	59.34	(9), (10)
7. Auro-isopropyl xanthate	$(CH_3)_2CHOCSSAu$	59.29	57.70	(9), (10)
8. Auro-*n*-butyl xanthate	$CH_3(CH_2)_3OCSSAu$	56.92	55.73	(9), (10)
9. Auro-isobutyl xanthate	$(CH_3)_2CHCH_2OCSSAu$	56.92	56.64	(9), (10)

*Supplied through the courtesy of the Schering Corporation, Bloomfield, N. J.
†Supplied through the courtesy of Mr. J. G. Bieri.
‡Supplied through the courtesy of Crookes Laboratories, Inc., New York, N. Y.
§Method of the American Medical Association.[11]

TOXICITY OF GOLD COMPOUNDS

The effects of gold compounds on the nonprotein nitrogen of the blood are given in Table II. The Anderson-Howell[12] method was used to determine all the nonprotein nitrogen values. Gold sodium thiosulfate was administered in water solution. The colloidal gold was a hydrosol. Water-insoluble strontium aurothioglycolate and the auroxanthates were administered intramuscularly in a sesame oil suspension. Since *α*-aurothio-*n*-butyric acid is soluble only to the extent of 54 mg. per 100 c.c. of water at 100° C., it also was administered in oil suspension. Gold sodium thiosulfate was injected as an aqueous solution.

Gold sodium thiosulfate is definitely toxic when administered in 5 mg. doses. In Animal 23 death occurred on the third day when the blood nonprotein nitrogen had reached a level of 248.1 mg. per 100 c.c. In Animal 24 the blood nonprotein nitrogen reached a level of 151 mg. on the fourth day but returned to normal on the seventh day. In animals receiving less than 5 mg. there was no appreciable rise in blood nonprotein nitrogen except in Animal 28, receiving 2 mg., where a value of 150.1 mg. was found on the second day after injection. This animal returned to normal on the third day.

α-Aurothio-*n*-butyric acid was toxic when administered at a 3 mg. level, one animal having a nonprotein nitrogen level of 339 mg. and dying the third day after injection. On the 5 mg. level one animal died the third day when the nonprotein nitrogen level had reached 185.4 mg. per 100 c.c. of blood. The three other animals receiving 5 mg. doses had high nonprotein nitrogen values the third day after injection. The fifth day after injection two of these were essentially normal, while the third had returned to normal the tenth day.

In the case of all the other compounds studied, there was no increase in nonprotein nitrogen level even after doses of 100 mg. in the cases of auro-*n*-butyl and auro-isobutyl xanthates.

TABLE II

SHOWING NONPROTEIN NITROGEN OF RAT BLOOD BEFORE AND AFTER INJECTING GOLD PREPARATIONS

ANIMAL	SEX	WEIGHT (GM.)	DOSE (MG.)	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)											
				BEFORE INJECTION	DAYS AFTER INJECTION										
					1	2	3	4	5	7	8	10	11	12	13
Gold sodium thiosulfate															
30	F	106	1	54.6	52.8	44.4	44.1			36.9					
29	F	139	1	39.1	37.1	46.2	36.6			43.2					
28	F	139	2	39.6	41.4	150.1	42.7			41.4					
27	M	167	2	39.4	46.5	45.6	53.2			48.6					
26	F	121	3	40.6	48.2	52.2	39.2			43.9					
25	F	113	3	41.0	42.8	44.1	45.3			45.3					
24	M	157	5	69.7	85.4	139.6	149.4	151.0		42.2					
23	M	114	5	43.9	136.4	156.2	248.1*								
α-aurothio-*n*-butyric acid															
35	F	169	3	42.4	96.3	175.6	339.0*								
32	M	185	3	38.1	35.5	34.3	47.4		37.9						
37	M	146	5	51.9	90.6	151.2	180.9		56.4			39.7			
36	F	167	5	51.1	95.9	92.9	131.6		37.3			43.9			
33	F	185	5	42.6	96.4	151.5	170.4		192.3			42.1			
31	M	211	5	40.4	131.7	176.4	185.4*								
Colloidal gold (Ruby)															
38	M	201	1.25	64.2	39.3	50.7		39.1							
39	F	177	2.25	42.4	36.3	37.8		41.2							
Strontium aurothioglycolate															
40	M	215	5	47.4	36.1	40.4	40.4								
41	M	202	14	33.4	43.0	39.9	41.4								
Auro-*n*-propyl xanthate															
44	F	141	5	35.9	35.9	35.6	34.0								
45	M	144	10	35.1	30.7	30.5	38.9								
46	M	241	15	46.4	33.6	48.3	34.4								
47	M	217	25	37.5	41.1	41.1	33.7								
Auro-isopropyl xanthate															
57	F	141	5	42.0	35.5	35.4	38.1	37.8							
56	M	233	10	36.7	39.3	47.1	36.1	43.9							
58	F	186	15	39.1	31.2	40.0	37.5	44.6							
59	M	238	25	37.1	40.7	40.4	40.4	37.4							
Auro-*n*-butyl xanthate															
48	M	174	10	41.6	30.7	37.0		36.9							
49	M	192	20	31.0	38.6	44.0		35.3							
50	F	190	50	40.8	40.0	40.3		51.5							
51	F	189	100	51.0	36.3	39.6		41.7					38.5	35.3	49.7
Auro-isobutyl xanthate															
52	F	203	10	40.5	34.0	36.3	42.9			46.5					
53	M	245	20	42.3	39.7	36.0	39.7	35.7		41.3					
54	F	183	50	30.7	30.7	35.6	38.5	43.2		38.3	36.8				
55	M	240	100	31.5	31.0	36.9	36.3	34.9							

*Died.

The results of this experiment indicate that the toxicity of gold compounds depends upon their solubility in water. Gold sodium thiosulfate and *α*-aurothio-*n*-butyric acid, which are water soluble, are toxic, whereas colloidal gold, strontium aurothioglycolate, and the auroxanthates, water-insoluble substances, are nontoxic, as indicated by their effects on the nonprotein nitrogen values of the blood. If a gold compound is toxic and the animal survives the dose given, the toxic reaction is temporary, the nonprotein nitrogen value of the blood returning to normal in from five to ten days after injection.

THE EFFECT OF TYPE OF CARRIER ON THE TOXICITY OF AUROTHIOGLUCOSE

Some of the compounds in the previous experiments were administered in oil suspensions and some were administered in aqueous solution. Many workers feel that administration of water-soluble aurothioglucose as an oil suspension makes this compound less toxic than when administered in aqueous solution. In order to determine the effect of type of carrier on the toxicity of aurothioglucose, animals were given intramuscular injections of varying doses in oil suspension and in aqueous solution. The effects of these injections on the nonprotein nitrogen level of the blood are given in Table III.

Table III

Showing the Effect of Type Carrier on the Toxicity of Aurothioglucose

ANIMAL	SEX	WEIGHT (GM.)	DOSE (MG.)	CARRIER	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)						
					BEFORE INJECTION	DAYS AFTER INJECTION					
						1	2	3	4	7	15
1	F	141	Control 1.0 c.c.	0.9% NaCl	53.3	43.5	50.7				34.6
10	M	185	Control 1.0 c.c.	Sesame oil		51.8	41.2	39.8		44.9	
2	M	161	10	Water	36.4	54.7	40.7	40.7		42.9	44.4
6	F	134	10	Oil	37.7	61.1	44.3	50.2		38.1	
4	F	143	40	Water	45.3	141.0	169.5	184.1		87.8	44.6
7	F	162	25	Oil	55.0	131.0	176.5	266.8*			
3	F	170	50	Water	51.8	101.0	197.5	151.5		81.2	44.2
8	M	180	50	Oil	60.0	126.0	214.2	205.6	363.6*		
5	M	184	100	Water	48.6	97.8	268.0	287.2	340.0*		
9	M	169	100	Oil	54.3	120.0	238.0	309.0		107.5	

*Died.

Table III shows a comparison of the effect of various doses or aurothioglucose on the nonprotein nitrogen level of the blood when administered in water solution and in oil suspension. There appears to be little difference in the toxicity of aurothioglucose as related to the mode of injection. On the 25 and 50 mg. levels, aurothioglucose appears to be more toxic when administered in oil than when administered in water solution, as indicated by the death of both animals receiving the oil suspension. On the 40 and 50 mg. levels in water solution both animals survived and were back to normal the fifteenth day. However, on the 100 mg. level death occurred in the animal receiving the water solution, whereas the animal receiving the oil suspension survived.

TOXICITY OF α-AUROTHIO-*n*-BUTYRIC ACID, DUE TO GOLD ATOM

In order to prove that the toxicity of gold compounds was due to the presence of gold, animals were given injections of α-thio-*n*-butyric acid and later α-aurothio-*n*-butyric acid. The effects of these compounds on the nonprotein nitrogen level of the blood are given in Table IV.

Table IV

Toxicity of α-Aurothio-*n*-Butyric Acid Due to Presence of Gold Atom

ANIMAL	SEX	WEIGHT (GM.)	α-THIO-*n* BUTYRIC ACID (MG.)	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)				α-AUROTHIO-*n*-BUTYRIC ACID (MG.)	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)				
				BEFORE INJECTION	DAYS AFTER INJECTION				DAYS AFTER INJECTION				
					1	2	3		1	2	3	5	10
31	M	201	50	36.9	41.3	47.8	40.4	5	131.7	176.4	185.4*		
32	M	181	25	48.4	41.4	38.1		3	35.5	34.3	47.4	37.9	
33	F	174	10	47.2	37.9	39.7	42.6	5	96.4	151.5	170.4	192.3	42.1
34	F	174	0.50 c.c. 0.9% NaCl	48.0	45.4	37.8	39.7	0.50 c.c. oil	41.4	42.3	42.3	43.9	

*Died.

Table IV indicates that α-thio-*n*-butyric acid, when administered in doses up to 50 mg., is nontoxic. When this is followed in the same animals by doses of 5 mg. of α-aurothio-*n*-butyric acid, definite toxicity results as indicated by a rise in blood nonprotein nitrogen values, and in one case by the death of the animal. It is evident from this that toxicity is due to gold.

DEVELOPMENT OF TOLERANCE TO AUROTHIOGLUCOSE AND GOLD SODIUM THIOSULFATE

Cortell and Richards[13, 14] studied the development of tolerance to gold salts in rats, using the survival method. They showed that rats given sublethal doses of gold sodium thiosulfate and of gold sodium thiomalate could tolerate ordinarily lethal doses of these compounds subsequently administered. They also showed that a rat which had developed tolerance to gold sodium thiomalate was also tolerant to gold sodium thiosulfate.

The results presented in Table V show the effects of repeated doses of aurothioglucose and of gold sodium thiosulfate on blood nonprotein nitrogen values. Rats given doses of 40 and 50 mg. of aurothioglucose showed a decided rise in blood nonprotein nitrogen values which returned to normal the fifteenth day. When the doses were repeated on these animals, blood nonprotein nitrogen values remained normal. A third dose of 75 and 100 mg. also showed no appreciable rise in the blood nonprotein nitrogen value. Animal 2, given an initial dose of 10 mg., a second dose of 20 mg., and a final dose of 50 mg., showed little change in blood nonprotein nitrogen values.

The results obtained with gold sodium thiosulfate although similar to those obtained with aurothioglucose are not as consistent. Seven rats were given initial doses ranging from 1 to 5 mg. Only two animals showed a rise in blood nonprotein nitrogen values, the one receiving 2 mg. and the other 5 mg. As a second dose each animal was given 5 mg. Apparently an initial dose of 1 mg. did not protect the animal since both of these animals died after the 5 mg. dose. Even initial doses of 2 and 3 mg. showed slight protection since in all cases the blood nonprotein nitrogen values were high, and in Animal 26, which had received an initial dose of 3 mg., death occurred. An initial dose of 5 mg. apparently protected the animal because there was little rise in the blood nonprotein nitrogen value after the second dose of 5 mg.

The four surviving animals were each finally given a 10 mg. dose. All animals survived and blood nonprotein nitrogen values were approximately normal on the seventh day after injection except in Animal 28. Animal 28

TABLE V

SHOWING DEVELOPMENT OF TOLERANCE TO AUROTHIOGLUCOSE AND GOLD SODIUM THIOSULFATE

ANIMAL	SEX	WEIGHT GM.		NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)							
				BEFORE INJECTION	DAYS AFTER INJECTION						
					1	2	3	4	7	9	15
AUROTHIOGLUCOSE											
			1st Dose mg.								
1	F	141	1 c.c. NaCl	53.3	43.5	50.7					34.6
2	M	161	10	36.4	54.7	40.7	40.7		42.9		44.4
3	F	143	40	45.3	141.0	169.5	184.1		87.8		44.6
4	F	170	50	51.8	101.0	197.5	151.5		81.2		44.2
			2nd Dose mg.								
1		150	0.5 c.c. NaCl		60.8	49.4	51.1			56.4	
2		212	20		47.1	41.7	53.0			52.4	
3		155	40		49.8	39.1	44.2			49.9	
4		175	50		44.7	42.2	48.4			53.8	
			3rd Dose mg.								
1		168	1 c.c. NaCl		48.7	50.7					
2		221	50		51.9	44.8	54.6				
3		159	75		48.9	41.6	47.5				
4		187	100		50.7	47.4	52.4				
GOLD SODIUM THIOSULFATE											
			1st Dose mg.								
29	F	139	1	39.1	37.1	46.2	36.6		43.2		
30	F	106	1	54.6	52.8	44.4	44.1		36.9		
27	M	167	2	39.4	46.5	45.6	53.2		48.6		
28	F	139	2	39.6	41.4	150.1	42.7		41.4		
25	F	113	3	41.0	42.8	44.1	45.2		45.3		
26	F	121	3	40.6	48.2	52.2	39.2		43.9		
24	M	157	5	69.7	85.4	139.6	149.4		42.2		
			2nd Dose mg.								
29		149	5		150.0	207.2	280.0*				
30		126	5		94.2	174.0	240.0*				
27		167	5		123.6	42.4	54.5		49.4		
28		139	5		104.8	116.2	153.8		100.0		
25		133	5		117.8	119.5	100.0		84.8		
26		141	5		86.0	209.0	175.6	255.6*			
24		157	5		78.3	47.1	39.4		49.8		
			3rd Dose mg.								
27		214	10		92.7	78.9	63.1		55.7		
28		146	10		137.4	197.8	255.4		159.6		
25		138	10		162.4	95.0	63.1		48.1		
24		193	10		105.2	132.2	85.0		44.1		

*Died.

appears to have been exceptionally sensitive to gold since the initial dose of 2 mg. produced an appreciable rise in the blood nonprotein nitrogen value.

The results of these experiments indicate that rats become more tolerant to gold compounds after having received sufficiently large initial doses of them.

DEVELOPMENT OF CROSS-TOLERANCE

Animals that had received aurothioglucose were injected with gold sodium thiosulfate and later with α-aurothio-*n*-butyric acid. Animals that had received gold sodium thiosulfate were injected with aurothioglucose and later with α-aurothio-*n*-butyric acid. Animals that had received auroxanthates were subsequently injected with aurothioglucose. The changes in the nonprotein nitrogen values of the blood following these injections are presented in Table VI.

TABLE VI

CROSS-TOLERANCE STUDIES

ANIMAL	SEX	WEIGHT (GM.)	GOLD SODIUM THIOSULFATE (MG.)	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)						α-AUROTHIO-*n*-BUTYRIC ACID (MG.)	NONPROTEIN NITROGEN IN BLOOD (MG. PER CENT)			
				BEFORE INJECTION	DAYS AFTER INJECTION						DAYS AFTER INJECTION			
					1	2	3	5	9		1	2	3	7
Aurothioglucose*-gold sodium thiosulfate-α aurothio-*n*-butyric acid														
3	F	181	10	52.4	98.0	76.9	49.6		42.7	10	62.1	41.8	44.6	40.0
Gold sodium thiosulfate†-aurothioglucose-α-aurothio-*n*-butyric acid														
			Aurothioglucose (mg.)											
27	M	183	100	55.7		49.9	87.3	50.0		10	61.1	55.8	43.1	
Auro-*n*-propyl xanthate†-aurothioglucose														
45	M	158	100	34.0	38.2	42.1	39.9	42.8						
Auro-*n*-butyl xanthate†-aurothioglucose														
49	M	214	50	35.3	50.6	51.7	56.6							
Auro-isobutyl xanthate†-aurothioglucose														
52	F	213	100	46.5	49.3	58.3								
Auro-isopropyl xanthate†-aurothioglucose														
58	F	179	50	44.6	77.0	112.8	125.0							
Controls—aurothioglucose														
64	M	186	50	49.4	102.3	132.4								
62	M	257	100	39.3	164.8	170.5								

*See Table II for effects of original doses.
†See Table I for effects of original doses.

Animal 3, which had originally received a dose of 50 mg. of aurothioglucose and whose blood nonprotein nitrogen value had reached 197.5 mg. per cent two days after injection, tolerated a 10 mg. dose of gold sodium thiosulfate. The blood nonprotein nitrogen value reached 98.0 mg. per cent one day after the gold sodium thiosulfate was given. Later this animal tolerated a 10 mg. dose of α-aurothio-*n*-butyric acid with very little rise in the blood nonprotein nitrogen value. Ten milligram doses of gold sodium thiosulfate and α-auro-*n*-butyric acid given to a normal rat are usually lethal.

Animal 27 originally received 2 mg. of gold sodium thiosulfate with little subsequent rise in blood nonprotein nitrogen values. When later given 100 mg. of aurothioglucose, the blood nonprotein nitrogen value rose to 87.3 mg. per cent three days after injection. Later this animal tolerated 10 mg. of α-aurothio-*n*-butyric acid with little rise in blood nonprotein nitrogen values.

As indicated in Table II, auroxanthates, even when given in 100 mg. doses, showed no toxicity as indicated by blood nonprotein nitrogen values. However, animals which had received auroxanthates show tolerance for subsequently administered aurothioglucose, as indicated in Table VI. Whereas

50 and 100 mg. doses of aurothioglucose show marked rises of blood nonprotein nitrogen values when given to normal animals, very little rise in blood nonprotein nitrogen values is noted when these doses are given to animals previously given auroxanthates. Of the auroxanthates studied, auro-isopropyl xanthate appears to be the only one that did not protect the animal against the toxic effect of subsequently administered aurothioglucose. Although the therapeutic value of auroxanthates has not been demonstrated, their effect in desensitizing animals is clearly demonstrated.

HISTOPATHOLOGIC STUDIES

Cortell and Richards[14] have found severe kidney damage in rats receiving gold sodium thiosulfate. Brown, Saleeby, and Schamberg,[15] in a study on rabbits, reported that marked injury to the kidneys may take place before definite blood changes occur. Block, Buchanan, and Freyberg[16, 17] have reported finding gold in the kidneys and other organs of the body following administration of various gold drugs.

In the studies here reported, autopsies were performed on all animals. Histopathologic studies of the kidney were made on several representative animals by Dr. W. T. S. Thorp, of the Animal Pathology Laboratory.

Insoluble gold compounds were found largely unabsorbed at the site of injection for as long as two weeks after administration. No evidence of the presence of the soluble compounds was noted. Nephritis was noted in most cases studied histologically. In all cases of nephritis a high blood nonprotein nitrogen value was noted. However, kidney damage was not observed in all cases where a high nonprotein nitrogen value occurred. From this it would appear that blood nonprotein nitrogen values serve as a more sensitive index of toxicity than the histologic study of the kidney.

SUMMARY

1. The nonprotein nitrogen value of the blood has been used in determining the toxicities of several commercial and experimentally synthesized gold compounds.

2. The water-soluble compounds, gold sodium thiosulfate, aurothioglucose, and slightly soluble α-aurothio-*n*-butyric acid, were found to be toxic.

3. The water-insoluble compounds, colloidal gold and auro-*n*-propyl, auro-isopropyl, auro-*n*-butyl and auro-isobutyl xanthates, were found to be nontoxic.

4. The toxicity of α-aurothio-*n*-butyric acid was found to be due to the gold portion of the molecule.

5. Tolerance and cross-tolerance to gold compounds were developed in animals.

6. High blood nonprotein values were always found where tissue changes indicating kidney damage had occurred. In some cases high blood nonprotein nitrogen values occurred where there was no evidence of tissue change.

REFERENCES

1. Møllgaard, H.: Chemotherapy of Tuberculosis, Copenhagen, 1924, Nyt Nordisk Forlag.
2. Forestier, Jacques: Rheumatoid Arthritis and Its Treatment by Gold Salts, J. LAB. & CLIN. MED. **20**: 827, 1935.
3. Towle, H., and Grund, J.: Progress in Dermatology, New England J. Med. **202**: 231, 1930.

4. Hartfall, S. J., Garland, H. G., and Goldie, Wm.: Gold Treatment of Arthritis, Lancet **233**: 784, 1937.
5. Sabin, A. B., and Warren, Joel: The Therapeutic Effectiveness of a Practically Nontoxic New Compound (Calcium Aurothiomalate) in Experimental, Proliferative Chronic Arthritis of Mice, Science **92**: 535, 1940.
6. Sabin, A. B., and Warren, Joel: The Curative Effect of Certain Gold Compounds on Experimental Proliferative Chronic Arthritis in Mice, J. Bact. **40**: 823, 1940.
7. Biilman, Einar: Studien über organische Thiosaüren, I, Ann. **339**: 351, 1905.
8. Biilman, Einar: Studien über organische Thiosaüren, II, Ann. **348**: 120, 1906.
9. Zeise, W. C.: Beiträge zur nahren Kenntnis und ihrer Verbindungen, Ann. **16**: 178, 1835.
10. Scala, A.: Su alcuni derivati dell' acido propilxantogenico, Gazz. Chim. Ital. **17**: 78, 1887.
11. American Medical Association: New and Nonofficial Remedies, 1940, Chicago, American Medical Association.
12. Anderson, Arthur K., and Howell, Stacey F.: The Determination of Nonprotein Nitrogen in One-Tenth of a Cubic Centimeter of Blood, J. LAB. & CLIN. MED. **16**: 183, 1930.
13. Cortell, Ruth, and Richards, R. K.: Development of Tolerance to Gold Salts in Rats, Proc. Soc. Exper. Biol. & Med. **49**: 121, 1942.
14. Cortell, Ruth, and Richards, R. K.: Studies on the Tolerance Formation to Gold Sodium Thiosulfate in Rats, J. Pharmacol. & Exper. Therap. **76**: 17, 1942.
15. Brown, H., Saleeby, E. R., and Schamberg, J. F.: Study of Toxic Effects of Certain Gold Compounds as Indicated by the Blood Chemistry and Pathological Changes in the Organs, J. Pharmacol & Exper. Therap. **28**: 141, 1926.
16. Block, W. D., Buchanan, O. H., and Freyberg, R. H.: Metabolism, Toxicity and Manner of Action of Gold Compounds Used in the Treatment of Arthritis, II, J. Pharmacol. & Exper. Therap. **73**: 200, 1941.
17. Block, W. D., Buchanan, O. H., and Freyberg, R. H.: Metabolism, Toxicity, and Manner of Action of Gold Compounds Used in the Treatment of Arthritis, IV, J. Pharmacol. & Exper. Therap. **76**: 355, 1942.

END OF
YEARS